Disclosure

Tamara MacDonald has received financial support for conference travel from Jazz pharmaceuticals, the manufacturer of Erwinia asparaginase.

Objectives

1. Discuss the minimum levels of L-asparaginase activity necessary to maintain L-asparagine depletion in children and young adults with ALL.
2. Describe differences in the pharmacokinetic and pharmacodynamic parameters between available asparaginase formulations.
3. Discuss results of clinical trials using different asparaginase formulations, with a focus on asparaginase depletion and clinical outcomes.
4. Discuss methods of incorporating asparaginase activity monitoring into clinical practice.
Asparaginase sources

- Animal
- Plants
- Fungus
- Actinomycetes
- Algae
- Bacteria
- Protein engineering or modified versions of the enzyme reduce hypersensitivity of native preparations

Asparaginase (ASP)

- Asparaginase hydrolyzes the amino acid L-asparagine to L-aspartic acid and ammonia.
- Asparagine is required for DNA synthesis and cell survival.
- Acute lymphoblastic leukemia (ALL) cells grow quickly and have rapid metabolic processes.
- ALL cells lack adequate levels of the required enzyme, asparagine synthetase, and require higher amounts so cannot survive asparagine depletion.

Mechanism of action of ASP

Blood L-asparagine

L-asparaginase

Leukemia cell does not produce L-asparagine due to low levels of asparagine synthetase

L-aspartate
Properties required of asparaginase product

- High binding affinity for its substrate asparagine (low Km value).
- Stable and active in pH of blood around 7.
- Low glutaminase activity.
- No adverse effects.
- No immunogenic complications.
- Delayed clearance from plasma (long half-life).

Asparagine depletion

- Asparaginase products deplete asparagine and glutamine, nonessential amino acids required for cell growth.
- Leukemia cells lack the ability to synthesize asparagine so rely on plasma asparagine to survive.
- Asparaginase products deplete plasma asparagine = leukemia cell death.
- Asparaginase products deplete plasma glutamine = non immunologic adverse effects.
- Glutamine can be used as an amino-group donor for asparagine.

Ways to measure asparaginase activity

- Serum asparagine levels:
  - Normal levels range from 40-80 uM and 0.1-0.2 uM considered complete depletion based on sensitivity of testing and NOT on formal criteria.
  - Critical level of asparagine depletion for leukemic cell death is not known.
  - Measured by high performance liquid chromatography.
  - Asparagine difficult to measure as degraded readily ex vivo.
- Anti-asparaginase antibody production:
  - Enzyme-linked immunosorbent assay IgG level shows active antibodies but difficult to measure and correlate or direct care.
- Asparaginase activity levels:
  - Inversely correlate with serum asparagine concentration
  - Validated level of activity and current reliable method of measuring asparaginase efficacy.
Minimum asparaginase activity levels required for asparagine depletion

- In 1981, Ricardi et al. administered *E. coli* and *Erwinia* asparaginase to rhesus monkeys and patients and found that plasma asparaginase activity levels above 0.1 IU/mL resulted in complete asparagine depletion in CSF and plasma.

*Ricardi, R., 1981;41:4554-8*

Therapeutic levels of asparaginase

- The cut-off of > 0.1 IU/mL has been confirmed and used in many clinical leukemia trials.
- Several trials have looked at a lower cut-off of 0.02-0.05 IU/mL but complete depletion is not consistent below 0.1 IU/mL.

*van der Sluis, IM., et al. 2016;100:279-285*

Question #1

- An asparaginase trough level of 0.2 IU/mL has been reported to you on a patient who has just received an asparaginase product. Which of the following would be your recommendation?
  A. Ask for an asparagine level to be drawn.
  B. Ask for a glutamine level to be drawn.
  C. Recommend that the next dose be given sooner than scheduled.
  D. Continue with the current treatment schedule and dose.
Marketed Formulations

Enzyme L-asparaginase are isolated either from *E.coli* or *Erwinia* chrysanthemi.

**Two *E.coli* products available**

- L-asparaginase (Kidrolase™): Available in Canada was marketed as Elspar™ in US.

*Erwinia* asparaginase (Erwinaze™)

### PK and PD data

<table>
<thead>
<tr>
<th>Type of ASP</th>
<th>n</th>
<th>Dose and route</th>
<th>ASP activity level</th>
<th>Conclusion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erwinaze</td>
<td>58</td>
<td>25,000 IU/m² M/W/F, IM</td>
<td>≥0.1 IU/mL most pts</td>
<td>Effective dose</td>
<td>Saluer 2013</td>
</tr>
<tr>
<td>Erwinaze</td>
<td>38</td>
<td>25,000 IU/m² twice weekly, IM</td>
<td>0.247 &amp; 0.077 IU/mL</td>
<td>Schedule for Erwinaze should be no longer than every 3 days</td>
<td>Vrooman 2010</td>
</tr>
<tr>
<td>Erwinaze</td>
<td>40</td>
<td>10,000 IU/m² daily for 2 x week IM</td>
<td>≥0.5 IU/mL</td>
<td>92% effective (OD)</td>
<td>Albertsen 2001</td>
</tr>
<tr>
<td>Erwinaze</td>
<td>21</td>
<td>20,000 IU/m² IM/W/F</td>
<td>0.16 IU/mL</td>
<td>48 hr level therapeutic</td>
<td>Vieira P 1999</td>
</tr>
<tr>
<td>Erwinaze/Na tive</td>
<td>58</td>
<td>10,000 IU/m² Native x1</td>
<td>Greater 3-day trough ASP activity level than Erwinaze</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>PEG-ASP</td>
<td>89</td>
<td>25,000 IU/m²/2 weeks, IV</td>
<td>Median 0.9 IU/mL</td>
<td>Dose reduction</td>
<td>Tong 2014</td>
</tr>
<tr>
<td>PEG-ASP</td>
<td>55</td>
<td>2000 IU/m² x 1 dose</td>
<td>Complete ASP depletion at 2h and 81% at 21 days half-life 7 days</td>
<td></td>
<td>Doser 2007</td>
</tr>
<tr>
<td>PEG-ASP</td>
<td>20</td>
<td>1000 IU/m² every 2 weeks x 2 doses</td>
<td>≥ 0.1 IU/mL</td>
<td>Adequate asparaginase depletion</td>
<td>Rizzari 2006</td>
</tr>
<tr>
<td>PEG-ASP/Native</td>
<td>118</td>
<td>2500 IU/m² every 2 weeks/6000 IU/m² M/W/F</td>
<td>≥ 0.03 IU/mL</td>
<td>More pts who received PEG-ASP resulted in higher ASP levels vs native E.coli</td>
<td>Dinndorf 2007</td>
</tr>
<tr>
<td>PEG-ASP/Native</td>
<td>118</td>
<td>2500 IU/m² every 2 weeks/6000 IU/m² weekly</td>
<td>PEG-ASP half-life 5.5 days and 26 hours for native E.coli</td>
<td></td>
<td>Avramis 2002</td>
</tr>
</tbody>
</table>
PK data for available formulations

<table>
<thead>
<tr>
<th></th>
<th>Erwinia asparaginase</th>
<th>Native E.coli asparaginase</th>
<th>PEG-ASP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Half-life</strong></td>
<td>IM: 16 hours</td>
<td>IM: 34-49 hours</td>
<td>IM: 7 days [naive]</td>
</tr>
<tr>
<td></td>
<td>IV: 7.5 hours</td>
<td>IV: 8-30 hours</td>
<td>IV: 6 days</td>
</tr>
<tr>
<td><strong>Asparagine depletion</strong></td>
<td>7-15 days</td>
<td>14-23 days</td>
<td>26-34 days</td>
</tr>
<tr>
<td><strong>Peak asparaginase activity</strong></td>
<td>Within 24 hours</td>
<td>24-48 hours</td>
<td>72-96 hours</td>
</tr>
<tr>
<td><strong>Dose, interval, route</strong></td>
<td>25,000 IU/m² IM, MWF (may need every other day IV)</td>
<td>6000 - 10,000 IU/m² IM three times weekly</td>
<td>1000-2500 IU/m² IV/IM every 2 weeks (may require lower dose)</td>
</tr>
</tbody>
</table>

Half-life of the agent may be shorter if previously exposed to a product isolated from the same organism or if a hypersensitivity reaction has occurred.

Question # 2

• AP was given a dose of 25,000 IU/m² *Erwinia* asparaginase IV on a M/W/F schedule and a trough level at 48 hours was 0.05 IU/mL after the second dose. What would you recommend?
  A. Switch to IM *Erwinia* asparaginase formulation and monitor levels.
  B. Increase the dose of the *Erwinia* asparaginase and monitor levels.
  C. Increase the frequency of administration and monitor levels.
  D. Increase the total number of doses of *Erwinia* asparaginase and monitor levels.

Factors that can impact asparaginase activity levels
Asparaginase Intolerance

- Suppression of the asparagine synthetase gene
- Production of antibodies against the drug (silent inactivation)
- L-asparaginase-sensitive cells produce cytokines that control the expansion of resistant cells
- Inactivation of caspase 3 enzyme.
- Cross sensitivity between E.coli products

Asparaginase Hypersensitivity Reaction

- Immune system reactions:
  - Depression of asparagine synthetase gene
  - Production of specific antibodies against the drug
  - Inactivation of caspase 3 or PARP (poly ADP-ribose) polymerase
  - Production of glutamine in large quantities by adipocytes

Mechanism of Hypersensitivity Reactions to Asparaginase Products

- Asparaginase products are derived from bacterial (or non-human) sources and as such have the potential to illicit an immune response and antibody production.
- Anti-asparaginase antibodies thought to be IgG and/or IgE mediated.
Outcomes of hypersensitivity reactions

- IgG mediated response may result in production of neutralizing antibodies with or without clinical signs of an allergic reaction (silent inactivation).
- IgE mediated response likely to result in mast cell degranulation and histamine release and possible subsequent anaphylaxis.
- Both can render the drug ineffective.

Incidence of hypersensitivity reactions

- Native *E.coli* asparaginase:
  - 30-70%
  - Higher with IV formulation
- PEG-ASP:
  - 2-50%
  - Several report higher with IV formulation
  - Higher with high risk ALL
  - Higher with post remission consolidation therapy
- *Erwinia* asparaginase:
  - 10-30% (in patients previously treated with native *E.coli*)
  - Higher with IV formulation

Factors that may impact hypersensitivity reactions/inactivation

- Weekly or every 2 weeks – continuous asparagine depletion vs 2-7 doses over 6 – 8 months as per COG protocols.
- Weekly (native *E.coli*) or every 2 weeks PEG-asparaginase dosing may overcome silent inactivation by desensitization.
- Every 6-8 weeks inactivation may continue (current COG protocols).
Clinical outcomes of asparaginase treatment protocols

**Postinduction dexamethasone and individualized dosing of E.Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: Results from a randomized Study DFCI Protocol 00-01.**

- 384 patients (median age 4.75 years), Fixed dose (n=195) and Individualized dose (n=189)
- Fixed dose (FD) native E.coli asparaginase 25,000 IU/m² vs Individualized dose (ID) starting at 12,500 IU/m² (median dose 17,500 IU/m²)
- Given IM weekly x 30 weeks
- Asparaginase activity level measured 7 days after 2nd and 4th dose and every 3 weeks.
- Asparaginase antibodies obtained every 6 weeks from all pts and when asp levels below detection 0.025 IU/mL.
- ID pts with asp levels less than 0.1 IU/mL on successive determinations despite dose adjustment or when asp antibodies present where considered to have silent inactivation and switched to Erwinia asp (25,000 IU/m² twice weekly) if available or PEG-ASP (not mentioned).
- FD pts only switched if clinical allergy present. 19/189 (10%) ID patients switched due to silent inactivation.


- Overall EFS higher in the ID vs FD group (90 vs 82%)
- No statistical difference based on age, gender, phenotype, cytogenetics or risk group.
- Conclusion: EFS better in ID group as patients who exhibited silent inactivation were switched to different asparaginase formulation.

Long-term results of a randomized trial on extended use of high dose L-asparaginase for standard risk childhood acute lymphoblastic leukemia.

- 355 randomly assigned patients with SR ALL
- Modified BFM backbone (less anthracycline and cyclophosphamide)
- Study group received native E.coli asparaginase 25,000 IU/m^2 x 20 weekly doses in continuation phase vs control group who did not receive any asparaginase in continuation.
- 5-year DFS 82% in the NO asparaginase group and 88% in the YES asparaginase arm.
- No significant differences in DFS based on age, gender or phenotype.
- Conclusion: more asparaginase in continuation for SR ALL on a modified (less intensive) BFM backbone increases survival significantly. When compared with the intermediate ALL group who received the non-modified BFM backbone the extra asparaginase did not result in increased survival.


Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01

- 352 patients (0-18 yrs)
- Randomization of PEG-asparaginase 2500 IU/m^2 IM every 2 weeks x 15 doses vs native E.coli asparaginase 25,000 IU/m^2 IM weekly x 30 doses during the intensification phase of therapy.
- 15% patients experienced allergic reaction.
- No significant difference in EFS allergy vs no allergy.
- 43 (12%) patients received less than 25 weeks of asparaginase due to toxicity.
- Asparaginase intolerance was associated with older age at diagnosis.
- 5-year EFS < 25 weeks vs at least 26 weeks of asparaginase is 73% vs 90%.
- Age, phenotype, presenting WBC count not predictors of EFS.
- Conclusion: Longer duration of asparagine depletion results in better survival.


Intravenous pegylated asparaginase versus intramuscular native Escherichia coli L-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia (DFCI 05-001): a randomized, open label phase 3 trial.

- 463 patients (1-18 years).
- IM native E.coli asparaginase 25,000 IU/m^2 weekly x 30 doses (n=231) or IV PEG-asparaginase 2500 IU/m^2 every 2 weeks x 15 doses (n=232)
- No difference in toxicity
- No difference in relapse incidence.
- No difference in 5-year DFS (p=0.52) including pts who did not receive all doses of asparaginase
- 5-year DFS allergic pts: 91% IV PEG-asparaginase vs 79% IM native E.coli asparaginase
- Asparaginase levels at 4,11, 18 days remained above 0.1IU/mL but below 0.1IU/mL at day 25 post PEG-ASP in 88% of pts.

Place, A., et al. 2015; 16:1677-90
Intravenous pegylated asparaginase versus intramuscular native Escherichia coli L-asparaginase in newly diagnosed childhood acute lymphoblastic leukemia (DFCI 05-001): a randomized, open label phase 3 trial.

- Patients with silent inactivation did not switch preparations.
- More pts in the IM native E.coli group had lower asparaginase levels at each sampling time point but no differences in DFS.
- Between weeks 11-29 all pts in the IV PEG-ASP group had levels > 0.1IU/mL suggesting that silent inactivation does not occur with repeated doses.
- Every 2 week schedule of 2500 IU/m² resulted in mean asparaginase level of 0.77 IU/mL suggesting that lower dose may be possible to maintain levels above 0.1IU/mL.
- When native E.coli ASP used in induction allergy incidence post induction was 23% vs Pts who received PEG-ASP IV in induction had 9% allergy incidence with native E.coli IM and 12% with IV PEG-ASP post induction.
- Conclusion: IV PEG-asparaginase no more toxic and just as efficacious as IM native E.coli asparaginase with reported better quality of life. Recommendation to give in induction.

Place, A., et al. 2015;16:1677-90

Effective asparagine depletion with pegylated asparaginase results in improved outcomes in adult acute lymphoblastic leukemia: Cancer and Leukemia Group B Study 9511.

- 85 patients (17-71).
- PEG-asparaginase 2000 IU/m² SubQ every 2 weeks.
- Age, WBC , phenotype were controlled for.
- Asparaginase antibodies and asparagine levels measured at several time points.
- Median OS improved from 13 months to 31 months for those who achieved asparagine depletion.
- Conclusion: PEG-asparaginase is efficacious and tolerated in the treatment of adult and adolescent and young adult (AYA) ALL.


Intensive high-dose asparaginase consolidation improves survival for pediatric patient with T cell lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study.

- 484 patients (1-21 years) with T cell ALL or advanced stage lymphoblastic lymphoma(n=244) vs control (n=240).
- All pts received 3 doses of 10,000IU/m² in induction and then randomized to native E.coli asparaginase 25,000 IU/m² IM weekly x 20 doses. Patients with allergy were switched to Erwinia at the same dose.
- Asparaginase arm had a significant increase in continuous complete remission rate. 5-year DFS 52.5% control vs 67.7% asparaginase.
- 59/244 (24%) pts reported allergy in study gp vs 24/240 (10%) on the control arm.
- Conclusion: asparaginase in continuation post-induction increase DFS at 5 years for T-cell and lymphoblastic lymphoma.

Recommendations based on clinical trial results

- Consistent monitoring of asparaginase levels to identify silent inactivation and modify therapy related to better event free survival.
- Increase asparaginase doses and asparagine depletion increases survival in less intensive leukemia protocols.
- Longer duration of asparagine depletion results in better survival.
- Longer duration of asparaginase treatment increases survival for both B and T cell leukemia.
- IV PEG-asparaginase should be given in induction therapy to decrease production of anti-asparaginase antibodies.
- PEG-asparaginase is efficacious in the treatment of adult and (AYA) ALL.

Question #3

SC is 21 years old and has relapsed ALL. He was treated with AALL1131 where he received 5 doses of PEG-ASP. What asparaginase therapy would SC best benefit from in his relapse ALL protocol?
A. Reduced number of asparaginase doses due to increased asparaginase toxicity in AYA.
B. Increased number of asparaginase doses due to better survival data in AYA.
C. Changing asparaginase products increases survival in patients with relapsed ALL.
D. Exclude asparaginase from the therapy protocol.

Factors that influence ASP activity

- The formulation of ASP
- Method of administration
- Degree of interpatient variability
- Formation of ASP antibodies
- Concomitant medications
Newer ASP formulation not yet marketed

- Calaspargase Pegol E.coli (SC-PEG) uses a succinimidyl carbonate linker which makes it more hydrolytically stable which results in higher asparaginase activity levels and longer asparagine depletion (18 vs 11 days) as compared to marketed PEG-asparaginase.
  

- Pegylated Erwinia longer half-life, Pegcrisantaspase, (Asparec®, JZP-416).

Method of administration

- IM half-life and length of asparaginase activity and asparagine depletion longer.
- IM less hypersensitivity or infusion related reactions.

Interpatient variability

- 416 patients received IV pegasparagase ASP activity levels were between 0.1-3.3 IU/mL
  

- A second study showed ASP activity levels ranged from 0.02- 0.5 IU/mL for 21 patients who received Erwinia asparaginase.
  
  Vrooman, LM., et al. 2013;31:1202-1210
Formation of ASP antibodies

- Native *E. coli* 60%.

- Pegaspargase 2-18%.

- *Erwinia* asparaginase 8-33%.

- Cross reactivity occurs between *E. coli* products:
  - 16 patients treated with native *E. coli*
  - 63% who then received pegaspargase developed antibodies
  - None who then received *Erwinia* developed antibodies

Concomitant medications

- Dexamethasone and asparaginase cause immunosuppression that may decrease anti-asparaginase antibody formation.
  Tong, WH., et al. 2014;123:2026-2033

Asparaginase monitoring

- Timing of level for accurate interpretation should be based on the half-life and documented length of asparagine depletion of each product.
- Asparaginase level above or equal to 0.1IU/mL or 100 IU/L is considered therapeutic.
- Several laboratories in the US that have validated asparaginase assays ex. AlBioTech Inc.
Current recommendation for sample timing for all patients to monitor for silent inactivation

- Native *E. coli* asparaginase: Asparaginase activity below the lower limit of laboratory quantification (LLQ) 72 hours post-dosing in a 2 x week schedule OR 7 days post dose in a weekly schedule.
- *Erwinia* asparaginase: Asparaginase activity below the LLQ 48 hours post dose M/W/F schedule (may do after the 2nd and 5th). More frequent evaluation of levels required with IV use as half-life shorter.
- PEG-ASP: 6-7 days after each dose for both IV and IM with gaps in dosing (may not be necessary after 26 weeks every two weekly dosing).

Current recommendation for sample timing for all patients who have clinical allergic reaction:

Allergies graded using CTC AE v4:
- Grade 1 (and mild Grade 2) reaction: PEG-ASP monitor serum asparaginase level within 7 days to identify inactivation
- Grade 2 (moderate-severe) to Grade 4: Switch asparaginase preparation

Changes recommended based on allergic reaction and/or the asparaginase levels

- Grade 1 or mild Grade 2: stop infusion and medicate with appropriate agent. If symptoms resolve restart the infusion and check level 6-7 days post infusion if full dose given. May check level 14 days post-dose to be assured of continued asparagine depletion.
- If level > 0.1 IU/mL premedicate and continue with same preparation and monitor for silent inactivation.
- If level < 0.1 IU/mL switch preparation
   - PEG-ASP/native *E. coli* switch to *Erwinia* IV or IM
   - Monitor *Erwinia* asparaginase levels and discontinue if level < 0.1 IU/mL.
   - If unable to switch preparation considered increasing the dose.
Question # 4

- RR is 3 years old with ALL and is being treated with AALL0932. After he received his first dose of PEG-ASP 2500 IU/m² IV he experienced a rash and some abdominal discomfort. He has one more dose of PEG-ASP IV. What would you recommend?
  A. Check an asparaginase level at day 7 post his first dose and if therapeutic premedicate and give the 2nd dose IV.
  B. Check an asparaginase level at day 7 post his first dose and if therapeutic switch to IM PEG-ASP.
  C. Check an asparaginase level at day 7 post his first dose and if subtherapeutic switch to Erwinia asparaginase IM (M/W/F).
  D. Check an asparaginase level at day 7 post his first dose and if subtherapeutic switch to Erwinia asparaginase IV twice weekly.

Research gaps

- Determination of minimal effective dose of asparaginase.
- Determination of the PK differences between IM and IV asparaginase and the evaluation of total number of doses required.
- Interval of administration based on length of asparagine depletion required.
- Optimal number of doses required for each protocol to optimize outcomes.
- Optimal dose of asparaginase required to overcome inactivation/intolerance.

Conclusion

- Monitoring of asparaginase activity levels is becoming standard of care to detect inactivation.
- The timing of the trough asparaginase activity levels is essential in therapy decisions.
- The route of administration alters the length of asparagine depletion.
- Therapy recommendations are limited to available data and based on product switching rather than dose and schedule adjustments.
- Appropriate grading of reactions is required to enable optimal therapy decisions.
References


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