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Clinical Study Protocol Guidelines

Guideline for Real-time Therapeutic Drug Monitoring (TDM) of asparaginase

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Real-time therapeutic drug monitoring (TDM) of asparaginase

Aim

Detection of silent inactivation (SI) and allergic like-reactions to improve asparaginase therapy

Background

Drug monitoring is the only method to detect *silent inactivation* of PEGasparaginase. Furthermore, by monitoring asparaginase levels we may prevent severe allergic reactions e.g. anaphylactic shock because the next dose will not be administered when silent inactivation is found. Patients with silent inactivation will switch to Erwinia asparaginase.
An *allergic-like reaction* is an intolerance or infusion reaction with e.g. vomiting, stomach ache, rash etc. These patients have normal activity levels if the infusion is continued. Real allergies often occur at the first drops, while allergic-like reactions occur later during infusion. Distinction between hypersensitivity and allergic-like reactions is critical but may be difficult.
Patients with an 'allergic-like reaction', mimicking a clinical allergy but without neutralization of asparaginase activity, might continue with the same preparation since they maintain adequate asparaginase activity levels when subsequent administrations are clinically possible.

Hypersensitivity definitions

PdL toxicity project definitions of hypersensitivity reaction will be used. (Reference: Schmiegelow et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol 2016.)

1. Allergy
2. Silent inactivation
3. Allergic-like reactions

Ad 1. allergy:

An adverse local or general response from exposure to Asp characterised by flushing, rash, urticaria, drug fever, dyspnoea, symptomatic bronchospasm, edema/angioedema, hypotension and/or anaphylaxis (accompanied by inactivation of Asp activity*).
** Trough level < Lower Limit of Quantification (LLQ) before the 'allergic' dose. A post-infusion Asp activity level cannot be measured accurately, when an infusion is stopped after a few millilitres.*

Ad 2. silent inactivation:

Patients without clinical allergy but with Asp activity levels, preferably measured in 2 independent samples, of:

PEGasp (biweekly schedule):	Day 7 < 100 and/or day 14 < LLQ
Erwinase(every other day schedule:	Day 2 < LLQ
(rec) E-coli asparaginase	Day 3 < LLQ
General definition:	(trough) Asp activity level < LLQ

Ad 3. allergic-like reaction:

An intolerance with e.g. vomiting, stomach ache, rash etc. These patients have normal activity levels if the infusion is continued. Real allergies often occur at the first drops, while allergic-like reactions occur later during infusion. Distinction between hypersensitivity and allergic-like reactions is critical but may be difficult.

Severity

mild: transient flushing or rash or drug fever < 38° C; or

severe: drug fever > 38° C; allergy-related edema/angioedema; dyspnea and/or symptomatic bronchospasm with or without urticaria, and/or hypotension and anaphylaxis) with indication for Asp infusion interruption and parenteral medication (e.g. antihistamines, glucocorticosteroids).

PEGasparaginase therapy and monitoring schedule

Asparaginase is administered in a continuous dosing schedule in post-induction in order to reduce the number of hypersensitivity reactions. Asparaginase activity levels will be measured in real-time in all patients to detect silent inactivation and distinguish allergic-like reactions from real allergic reactions. Patients will switch preparation in case of inactivation. Inactivation (allergy and silent inactivation) can occur following any dose and will occur more frequently in the first doses of PEGasparaginase administration in post-induction and after a dose interruption.

PEGasparaginase administrations and monitoring are summarized in table 1 to 4.
PEGasparaginase is administered IV or IM in a **fixed dose of 1500 IU/m² in patients < 16 years of age and 1000 IU/m² in patients ≥ 16 years of age**. All patients < 25 years of age receive 2 doses in induction (day 4, 18). Patients ≥25 years will receive one dose (day 18) in induction, the omitted dose from day 4 is rescheduled and administered after the last dose. The number of total doses depends on the risk group stratification. Patients in the standard risk group receive a total of 4 doses, in the intermediate risk (IR)-low 5 doses, and the IR-high 8 doses. High Risk group patients receive 1 to 10 doses. HR blocks contain 1 PEGasparaginase dose per block and 2 to 3 doses in delayed intensification. Because of the discontinuous dosing schedule in the HR blocks week levels will be measured to detect silent inactivation.

Table 1. PEGasparaginase doses and **minimal** TDM guidelines in SR and IR groups (<25 yrs)

Phase	PEGasp		Asparaginase activity Sampling
	number	Day	
	SR (4 doses)		
Induction	1 -- 2 --	4 11 18 32	- Yes (week level) yes, before infusion yes
Cons 2	3 -- 4 --	71 78 85 99	- yes (week level) yes, before infusion yes
	IR low (5 doses)		
Induction	1 -- 2 --	4 11 18 32	- Yes (week level) yes, before infusion yes
Cons 2	3 -- 4 5	78 85 92 106	- yes (week level) yes, before infusion yes, before infusion
	IR high (8 doses)		
Induction	1 -- 2 --	4 11 18 32	- Yes (week level) yes, before infusion yes, before infusion.
Cons 2	3 - 4 5 6	78 85 92 106 120	- yes (week level) yes, before infusion yes, before infusion -
DI	7 8	134 148	yes, before infusion -

See guidelines for additional sampling below table 4

Table 2. PEGasparaginase doses and **minimal** TDM guidelines in SR and IR groups (≥ 25 yrs)

Phase	PEGasp		Asparaginase activity Sampling
	number	Day	
	SR (4 doses)		
Induction	1	18	-
	-	25	Yes (week level)
	-	32	Yes
Cons 2	2	71	-
	--	78	yes (week level)
	3	85	yes, before infusion
	4	99	yes , before infusion
	IR high (8 doses)		
Induction	1	18	-
	--	25	Yes (week level)
	-	32	yes
Cons 2	2	78	-
	-	85	yes (week level)
	3	92	yes, before infusion
	4	106	yes, before infusion
	5	120	-
DI	6	134	yes, before infusion
	7	148	-
	8	162	yes, before infusion

See guidelines for additional sampling below table 4

Table 3. PEGasparaginase doses and minimal TDM guidelines in the HR group (<25 yrs).

Phase	PEGasp dose		Asparaginase activity Sampling
	Number	Day	
	HR (2-10 doses)		
Ind	1	4	-
	-	11	yes (week level)
	2	18	yes, before infusion
Cons I	-	32	yes
HR A1	3	A1 day 6	-
	-	A1 day 13	yes (week level)
HR B1	4	B1 day 7	-
	-	B1 day 14	yes (week level)
HR C1	5	C1 day 7	-
	-	C1 day 14	Yes (week level)
HR A2	6	A2 day 6	-
	-	A2 day 13	yes (week level)
HR B2	7	B2 day 7	-
	-	B2 day 14	yes (week level)
HR C2	8	C2 day 7	-
	-	C2 day 14	yes (week level)
maintenance	-	-	-
DI	9	309 (day1 of DI)	-
	-	316	yes (week level)
	10	323	yes, before infusion

See guidelines for additional sampling below table 4

Table 4. PEGasparaginase doses and minimal TDM guidelines in the HR group (≥ 25 yrs)

Phase	PEGasp dose		Asparaginase activity Sampling
	Number	Day	
	HR (1-10 doses)		
Ind	1	18	-
	-	25	yes (week level)
Cons I	-	32	yes
HR A1	2	A1 day 6	-
	-	A1 day 13	yes (week level)
HR B1	3	B1 day 7	-
	-	B1 day 14	yes (week level)
HR C1	4	C1 day 7	-
	-	C1 day 14	Yes (week level)
HR A2	5	A2 day 6	-
	-	A2 day 13	yes (week level)
HR B2	6	B2 day 7	-
	-	B2 day 14	yes (week level)
HR C2	7	C2 day 7	-
	-	C2 day 14	yes (week level)
maintenance	-	-	-
DI	8	309 (day1 of DI)	-
	-	323	yes (week level)
	9	323 (day 15)	yes, before infusion
	10	337	-

NOTE 1: More frequent asparaginase activity sampling is encouraged!:

- **Allergic or allergic-like reaction: take an extra (peak) level**, especially when no trough level is available (see switching guideline). Register how much asparaginase was administered.
- Reintroduction of PEGasparaginase (e,g, caused by toxicity): monitor after 1 week and 2 weeks to detect SI
- Declining/low activity levels to detect silent inactivation
- Silent inactivation should be confirmed in 2 independent samples. Take the second sample as soon as possible after obtaining knowledge of suspected SI
- If extra samples are taken it is advised to take day 7 and/or day 14 levels

NOTE 2: Consider storage of a pre-treatment serum sample for the assessment of anti-drug-antibodies and storage of every serum sample measured.

NOTE 3: Peak levels (within 1 hour after infusion) are also measured, after written informed consent, in patients participating in the asparaginase-outcome study (see for detailed sampling schedule **add-on study** protocol "Association between asparaginase activity levels and outcome").

Table 5 PEGasparaginase doses and **minimal** TDM guidelines in Down syndrome patients

Phase	PEGasp		Asparaginase activity sampling
	number	Week/day	
	Down SR (4 doses)		
Induction	1 2 -	Day 4 Day 18 -	Day 11 (week level) Day 18 before infusion Day 32
Interim maintenance	3 4 -	Week 11 day 71 Week 13 day 85 -	day 78 (week level) Day 85 before infusion Day 99
	Down IR (5 doses)		
Induction	1 2 -	Day 4 Day 18 -	Day 11 (week level) Day 18 before infusion Day 32
Capizzi	3 4 -	Week 12 day 80 Week 14 day 94	Day 87 (week level) Day 94 before infusion Day 108
Delayed intensification	5 -	Week 20 day 134 -	Day 141 (week level) Day 148
	Down HR (8 doses)		
Induction	1 2 -	Day 4 Day 18 -	Day 11 Day 18 before infusion Day 32
HR Consolidation 1 (Augmented BFM)	3 4	Week 8 day 51 Week 12 day 79	Day 58 (week level) Day 86 (week level)
Capizzi	5 6	Week 16 day 108 Week 18 day 122	Day 115 (week level) Day 136
Delayed intensification	7 8	Week 24 day 165 Week 30 day 204	Day 172 (week level) Day 211 (week level)

Switching guidelines PEG- and Erwinia asparaginase

1. Allergy all grades *and* inactivation

a. Switch PEGasp to Erwinia asp (20.000 U/m² IV or IM, every other day).
If the patient also develops an allergy with inactivation to Erwinia asp, no alternative asparaginase preparation is available and asp treatment needs to be truncated.
Consider switch to native *E-coli* asparaginase (e.g. Spectrila® 5.000 IU/m2, IV or IM every 3 days) with an allergy to the first PEGasparaginase dose, because this often is a PEG allergy and not an *E-coli* asparaginase allergy. Also consider an allergic-like reaction.
b. Do not use steroids or antihistamines to administer next doses, because it will not reverse the inactivation.
c. When no trough asparaginase activity level is available *see 3b*. If clinically impossible to re-expose the preparation should be switched or stopped.

2. Silent inactivation

a. Switch PEGasp to Erwinia asp (20.000 U/m² IV or IM every other day)
Consider switch to native *E-coli* asparaginase (e.g. Spectrila® 5000 IU/m2 IV or IM every 3 days) with SI of the first dose, because this often caused by anti-PEG antibodies and not by anti- *E-coli* asparaginase antibodies.

b. If the patient also develops an allergy to or SI of Erwinia asp, no alternative asparaginase preparation is available and asparaginase treatment needs to be truncated.

3. Allergic-like reaction

a. When the trough asp activity level taken before this dose is adequate you can re-expose carefully to the same preparation (if clinically possible). In these cases premedication with hydrocortisone and antihistamines is allowed as well as decreasing the infusion rate. Re-exposure should only be performed in the context of strict monitoring of activity levels (see b.)

b. When no trough level is available, check the asparaginase activity level after the truncated dose taking into account how much of the dose is administered, in case of detectable asparaginase activity you can re-expose carefully to the same preparation (if clinically possible).
(guidelines for asparaginase activity level measurement: after PEGasp immediately (within one hour = peak level) after the truncated dose, within a few days or 1 week. Timing of asp activity level also depend on how much of the dose is given! In case of Erwinia asp take a peak level and a level after 24-48hrs. If only a few milliliters are infused a post-dose activity level might not be informative and asparaginase preparation should be switched or stopped.)

Erwinia asparaginase therapy and monitoring schedule

Patients with a clinical allergy (with inactivation) or silent inactivation of PEGasparaginase will switch to Erwinia asparaginase. Type of allergy and grading according to PdL definitions and interventions (medication, i.v. saline, how much asparaginase has been administered etc.) is documented. This documentation is also needed in case of an allergy to Erwinia asparaginase.

Dose and timing

- The dose of Erwinia asparaginase is 20.000 IU/m^2 IV or IM every other day
- Start Erwinia asparaginase ASAP(within a few days) after the occurrence of the allergic reaction or SI, do not wait for 2 weeks (because activity levels will be <LLQ) , and replace the last PEGasparaginase dose.
  Substitution of 1 dose PEGasparaginase by 7 doses Erwinia asparaginase.

Aims TDM of Erwinia asparaginase:

- To detect SI
- To distinguish allergic-like reactions from real allergic reactions

TDM time points/frequency of monitoring

- Sampling should be started immediately, **before** the first Erwinia asparaginase infusion (to check PEGasparaginase activity/necessity to switch)
- Prior to the 1st, 2nd, 4th, and 6th Erwinia asparaginase infusion: asparaginase activity level
- Send the first 4 asparaginase levels (batch wise) to the laboratory
- Repeat asparaginase level every 2 weeks (1x T48 level)

- Reintroduction of Erwinia asparaginase after an Erwinia asp-free interval (e.g. in cons2 or HR blocks):
  Asparaginase activity level prior to 2nd and 6th Erwinia asparaginase infusion
  Repeat asparaginase level every 2 weeks (1x T48 level)

- In case of a suspected allergic -like reaction take a peak level (within one hour) and a 24-48 hour asp activity level as described above (section 'Switching guidelines PEG- and Erwinia asparaginase') .
  Continue erwinia asp when clinically possible.
- Stopping guidelines:
  - Silent inactivation
  - (real) allergies with inactivation

NOTE 1: Erwinia asparaginase clearance decreases in the first weeks of treatment
NOTE 2: 1 vial of Erwinia asparaginase contains 10.000 U. Adapting a dose can only be based on asparaginase activity levels, in that case dose can be rounded to the nearest whole vial strength.

NOTE 3:The dosing schedule of Erwinia asp can only be adapted when trough levels ≥ 100 U/L are ensured for all dosing intervals. More frequent monitoring will be needed, e.g. T48 *and* T72 in a M-W-F schedule.

(Recombinant) native E-coli asparaginase

Very few patients will receive native E-coli asparaginase. Also, commercially available as recombinant E-coli asparaginase (Spectrila®). Native E-coli asparaginase can be considered after an allergy to or silent inactivation of the **first** PEGasparaginase dose, because this often is a PEG allergy and not an E-coli asparaginase allergy. Native E.coli asparaginase could also be considered if only anti-PEG antibodies, but no anti-(*E.coli*)-asparaginase antibodies are detected after an allergy or silent inactivation later during treatment.

- Dose: Native E-coli asparaginase or Spectrila® 5000 IU/m^2 IV or IM, every 3 days
- Asp activity monitoring: prior to the first, 2nd and 3rd dose
  Adequate asparaginase level (≥ 100 U/mL)?
  - yes, take a trough level every other dose
  - no, switch to Erwinia asparaginase
- Allergy (with inactivation) switch to Erwinia asparaginase

Method of asparaginase activity level measurement

Asparaginase activity levels will be assessed in centralized laboratories in the various countries using the L-aspartic β-hydroxamate (AHA) test.(22) AHA is hydrolyzed by asparaginase to L-aspartic acid and hydroxylamine. Hydroxylamine will be diluted with 8-hydroxiquinoline for condensation and oxidation and quantified by photometric detection.
Quality control of the asparaginase activity measurements is assured by a cross validation process (distributing samples, receiving results, setting up statistics, response to the labs) performed by an independent institute Referenzinstitut für Bioanalytik (https://www.rfb.bio/). This institute is certified by the German Medical Association.

Asparaginase activity levels will be measured in all patients as standard of care and will be captured in the ALLTogether database.