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LETTER TO THE EDITOR

The optimal use of PEG-Asparaginase in relapsed ALL—lessons from the ALLR3 Clinical Trial

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Though modern chemotherapy regimens cure over 80% of children with acute lymphoblastic leukaemia (ALL), the outcome of relapsed disease remains suboptimal. Current curative strategies for relapsed patients are based on high dose cytotoxic therapy often followed by an allogeneic stem cell transplant. Drug-related toxicities limits the intensification of therapy and can result in treatment delays as well as deaths in remission. The drug L-Asparaginase (ASNase) is not associated with a dose-limiting toxicity and has been widely used not only in strategies for relapsed ALL[1] but also in early phase clinical trials.[2] ASNase is a bacterial protein and the main disadvantage of its repeated use is sensitization leading to clinical hypersensitivity reported in up to 60% or silent inactivation of the drug in 30% of patients receiving native *Escherichia Coli*–ASNase.[3] As most patients receive intensive ASNase during frontline therapy, there is then the question of its continued efficacy on subsequent use in relapsed patients. One option is the use of the noncross reacting Erwinia product, but this has a shorter half life and is prohibitively expensive. The polyethylene-conjugated ASNase (PEG-ASNase) is associated with less antigenic reactions, as a lower dose is required and it can be given less frequently. It is thus increasingly being used in both frontline and relapsed-therapeutic protocols. The optimal dose remains unclear and the drug is administered ranging from 2500 IU m^{-2} (refs. 2, 4) (weekly) to 1000 IU m^{-2}(ref. 1) (fortnightly). Before 2003, all *de novo* childhood ALL patients in UK received native ASNase. Since then patients have received PEG-ASNase, 1000 IU m^{-2} given intramuscularly, twice in induction and once in delayed intensification with high-risk patients receiving up to an additional nine doses.[5] Relapsed patients in the international ALLR3 trial (2003–2013, ISCRTN45724312) were given the same dose of PEG-ASNase, two doses in induction and once during consolidation[1] (Supplementary Figure 1). To assess the effect of PEG-ASNase given during initial therapy on its subsequent use in relapse patients, we recruited after obtaining ethical approval and written consent, patients on the ALLR3 clinical trial who had previously been treated on the UKALL 2003 protocol. Thirty-three patients were analysed between Jan 2009—May 2011. Trough ASNase activity was measured in plasma samples 7–14 days after each PEG-ASNase dose. ASNase activity was measured using a modified indoxine method.[6] A trough level of $\geqslant$100 IU/l was taken to represent adequate therapeutic activity.[7] Antibodies against PEG-ASNase and native *E. coli*–ASNase (Asparaginase Medac) were measured by indirect enzyme-linked immunosorbent assays developed and validated at Medac GmbH (Hamburg, Germany) from samples taken for activity analysis and in a sample obtained once ASNase therapy was completed but before transplantation (Supplementary Figure 2). Of the 33 patients recruited (Table 1), a total of 21 patients had ASNase activity measured at least at one time point (maximum of three). Only one patient did not show adequate activity with the rest demonstrating activity $\geqslant$200 IU l^{-1} (Supplementary Figure 2). We were able to serially monitor nine patients. With the exception of the previously described patient with inadequate levels, the other eight were found to have adequate activity at more than one time point. Antibody assays were performed in 19 of the 21 patients with ASNase activity results and an additional 14 patients in whom we did not have activity data. Only one of the 33 patients was detected to have antibody against both PEG-ASNase and native ASNase. This was the same patient who did not have adequate ASNase activity levels. Thus 1000 IU m^{-2} of PEG-ASNase given intramuscularly provides adequate therapeutic levels in patients who relapse of a PEG-ASNase frontline protocol. For patients treated initially with PEG-ASNase on frontline protocols who subsequently relapse, silent inactivation does not appear to pose a significant clinical problem.

At the time these assays were performed, 390 patients, recruited from participating centres in UK, Netherlands, Australia and New Zealand, had completed the first three blocks of therapy containing ASNase (Supplementary Figure 1).[1] Patients recruited to ALLR3 with hypersensitivity to *E Coli*-derived ASNase (either native or PEG) received the *Erwinia Chrysanthemi* derivative Erwinase. We identified 354 patients who received PEG-ASNase, 20 who received Erwinase and 16 patients who received no ASNase in the ALLR3 trial (Table 1). For a period of time, Erwinase was not available and patients with hypersensitivity ($n=14$) did not receive any ASNase. Similarly, patients who developed pancreatitis ($n=2$) during frontline therapy did not receive any ASNase in ALLR3. We examined the impact of not receiving ASNase on the outcome of these relapsed patients. Progression-free and overall survival were analysed using the Kaplan–Meier plot (unstratified) and log rank test. The 3-year progression-free survival and overall survival of the 16 patients who did not receive ASNase were 42.9 (95% CI 30.3,55.5) and 49.2% (95% CI 36.5,61.9). This was not significantly different from the progression-free survival of 46.4% (95% CI 35.4,57.1) ($P=0.377$) and overall survival of 53.5% (95% CI 41.8,63.9) ($P=0.365$) of the 354 patients who received PEG-ASNase (The 20 patients who received Erwinase when it became available at a later date have not been analysed here). The outcome of relapsed patients is related to the duration of first remission, site of relapse and immunophenotype,[8] with late isolated extramedullary and progenitor-B-cell phenotype having the best outcomes. In this latter category, there were no patients who had not received ASNase. To eliminate bias, a matched case-control analysis with a 3:1 ratio including all non-PEG patients and the maximum number of PEG patients randomly selected to obtain balance in both groups (same percentages) was performed. The progression-free survival and overall survival in the PEG and non-PEG groups were not significantly different (Figure 1). Unlike that previously reported for patients treated on frontline protocols,[9] survival was similar for patients with relapsed ALL irrespective of whether they received ASNase or not. A recent report from the frontline ALL2003 trial has also failed to show a difference in outcome in patients with ASNase-induced pancreatitis who received no further ASNase.[10]

Of the 354 patients who received PEG-ASNase as part of their relapse therapy, 241 and 113 patients, respectively had received native or PEG-ASNase during frontline treatment. Twenty-two (6%)

Table 1. Distribution of patients in the study

	PEG-ASNase		*None*		*Assayed patients*	
n	354	%	16	%	33	%
Sex						
Male	224	63.3	9	56.3	24	72.7
Female	130	36.7	7	43.8	9	27.3
Age at first relapse (years)						
Median (IQR)	9.3 (6.9–13.3)		8.3 (5.9–11.3)		8.6 (7–11.4)	
<10	193	54.5	11	68.8	24	72.0
⩾10	161	45.5	5	31.3	9	27.3
Immunophenotype						
B cell	302	85.3	14	87.5	27	81.2
T cell	52	14.7	2	12.5	6	18.2
Time to relapse (months)						
Median (IQR)	39 (25–55)		30 (19–42.5)		34 (18–48)	
Very early	46	13.0	4	25.0	8	24.2
Early	117	33.1	7	43.8	10	30.3
Late	191	54.0	5	31.3	15	45.5
Site of relapse						
Isolated extramedullary	89	25.1	0	0.0	14	42.4
Isolated marrow	205	57.9	11	68.8	15	45.5
Combined	60	16.9	5	31.3	2	6.1
Risk group						
Standard	21	5.9	0	0.0	3	9.1
Intermediate	234	66.1	8	50.0	17	51.5
High	99	28.0	8	50.0	13	39.4
Cytogenetics						
High hyperdiploid	54	15.3	6	37.5	8	24.2
ETV6-RUNX1	37	10.5	1	6.3	2	6.1
Intermediate	68	19.2	3	18.8	10	30.3
Poor	39	11.0	3	18.8	1	3.0
Unknown	156	44.1	1	6.3	12	36.4
Outcome						
Alive and well	184	52.0	7	43.8	16	48.5
Refractory to treatment	25	7.1	3	18.8	2	6.1
Second relapse	74	20.9	3	18.8	4	12.1
Disease-related death	10	2.8	0	0.0	0	0.0
Treatment-related death	40	11.3	2	12.5	6	18.2
Withdrawn	19	5.4	0	0.0	4	12.1
Lost to followup	1	<1	0	0.0	0	0.0
Second malignancy	1	<1	1	6.3	1	3.0

Abbreviation: IQR, interquartile range.

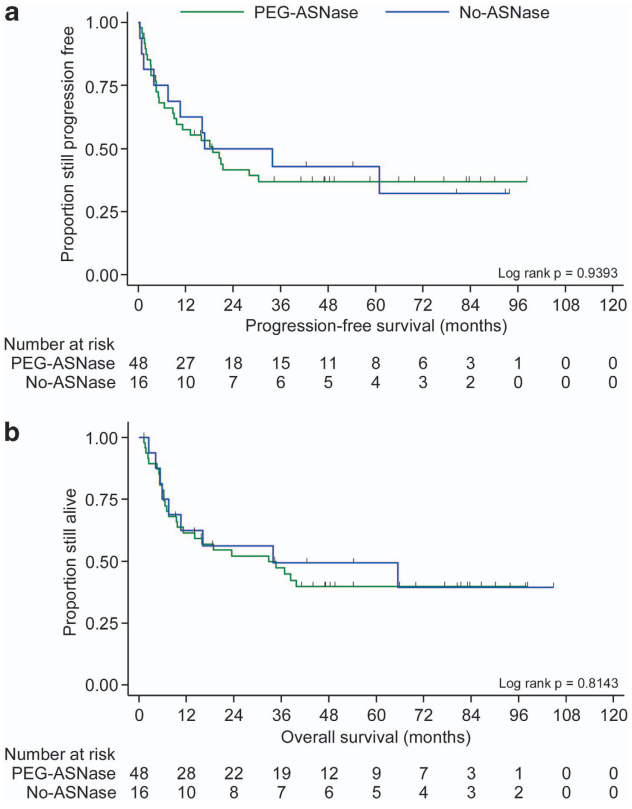

Figure 1. Kaplan–Meier estimates of (**a**) progression-free and (**b**) overall survival in relapsed ALL patients who received no asparaginase ($n = 16$) and a 3:1 risk-stratified matched cohort of those who received PEG-Asparaginase (ASNase) ($n = 48$).

patients, 12/241(5%) who had received native ASNase and 10/113 (9%) who had received PEG-ASNase in frontline therapy reported grade 3-4 toxicities associated with ASNase. Ten developed pancreatitis, seven had thrombosis, four had encephalopathy and one had hypersensitivity. The toxicity data for patients in ALLR3 who received *E. Coli*–ASNase or PEG-ASNase in the frontline protocol are comparable, though the slightly lower incidence with previous native ASNase may reflect the expected higher incidence of silent antibodies. PEG-ASNase at this dose can be administered at relapse, with no excess toxicity in patients who have previously received *E. Coli*–ASNase in frontline therapy.

The numbers analysed here are small, a reflection of the rarity of the disease, and thus interpretations of this data must be viewed cautiously. However we feel that our data will inform consortiums in the rational design of therapeutic strategies incorporating ASNase for those with recurrent ALL. This includes optimising both the dose and timing of ASNase. For patients relapsing of a frontline protocol using PEG-ASNase exclusively, the drug may be used again safely and a dose of 1000 IU m^{-2} appears to be adequate for most patients. Furthermore, as in ALLR3, the timing of PEG-ASNase administration can be optimised to potentially maximise the effect of dexamethasone,[11] as well as high dose methotrexate and cytarabine blocks in a Capizzi design.[12] All patients in this study analysed for ASNase activity relapsed of the UKALL 2003 trial and received 3-12 doses (based on risk stratification) of PEG-ASNase at a dose of 1000 IU m^{-2}.[5] Thus we do not have comparable activity data from patients who initially received *E. Coli*–ASNase in earlier frontline protocols, though we found no differences in toxicity. The use of PEG-ASNase after *E. Coli*–ASNase has been previously reported to be associated with a high incidence of hypersensitivity and drug inactivation owing to the presence of antibodies.[13] However if antibody titres are low, PEG-ASNase may still provide adequate activity levels.[14] A dose of 2500 IU m^{-2} given weekly has been shown to provide therapeutic levels in relapsed ALL patients who have previously received *E. Coli*–ASNase.[15] The monitoring of ASNase activity/antibody and increasing the dose of PEG-ASNase in those who have inadequate activity/silent antibodies could optimise drug administration and lead to a better outcome. This merits investigation in future trials for relapsed ALL.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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