Human leucocyte antigen alleles related to asparaginase allergy in children with acute lymphoblastic leukaemia

Inez M. Bérénos*[a], Vincent Gagné[a], Thai H. Tran[a], Yves Théorêt[a] & Maja Krajinovic[a]
[a]Charles-Bruneau Cancer Center, CHU Sainte-Justine Research Center, University of Montreal, Montreal, Quebec, H3T1C5, Canada
*Correspondence: i.m.berenos@students.uu.nl

Aim: To determine the association between allele variants coding for the human leucocyte antigen (HLA) and pegylated L-asparaginase (PEG-ASNase) induced allergy in children with acute lymphoblastic leukaemia (ALL) treated according to the Dana-Farber Cancer Institute (DFCI) 16-001 protocol. **Patients and methods:** Patients aged 1-22 years with newly diagnosed ALL were genotyped. DNA was extracted from serum samples and the presence of the HLA-DRB1*07:01 allele was determined using PCR analysis. For patients harbouring this allele, an additional two-step PCR test was done to determine presence of HLA-DQB1*02:02. **Results:** DRB1*07:01 carriers did not have a significantly higher risk of experiencing allergy compared to non-carriers (OR=1.1; p>0.05). Similarly, the DRB1*07:01/DQB1*02:02 haplotype did not increase allergy risk (OR=1.4; p>0.05). However, the association between allergies graded three and higher and the presence of DRB1*07:01/DQB1*02:02 did result in a greater OR and p-value closer to statistical significance (OR=2.9; p=0.15) **Conclusion:** This study was not able to demonstrate an increased risk of PEG-ASNase allergy in patients positive for HLA allele variants.

Keywords: acute lymphoblastic leukemia; pharmacogenomics; asparaginase; asparaginase hypersensitivity; asparaginase allergy; human leukocyte antigen

Acute lymphoblastic leukaemia (ALL) is a type of cancer that affects the blood and bone marrow. This disease causes the bone marrow to produce large quantities of immature lymphocytes, consequentially decreasing the number of functional lymphocytes, red blood cells and platelets in the body. While ALL mostly occurs in healthy patients, polygenetic and environmental risk factors have been reported (1). About 26% of all cancer cases in children under the age of 15 are diagnosed as ALL, making this the most common paediatric cancer with the highest incidence in children between two and four years old (2). Outcome has improved steadily over the past decades. Currently, the five-year survival rate has reached 90% for children in the lowest ALL risk group in developed countries (1,2). This improvement is the result of a combination of advances in molecular biology, innovations in therapeutics, use of personalized and evident-based medicine, and attention for supportive care (3). In addition, pharmacogenomic approaches are increasingly used to further personalize therapy by using genetic markers to predict patients' reactions to drugs (3,4).

The chemotherapeutic L-asparaginase (ASNase) is administered during multiple phases of ALL treatment. Its mechanism of action is primarily based on deamination of serum asparagine, resulting in the reaction products aspartic acid and ammonia (figure 1) (5,6). In addition to asparagine, glutamine concentrations are also reduced, contributing to the observed therapeutic effect. Due to the delayed expression of asparagine synthase, leukemic cells do not produce adequate amounts of asparagine and glutamine *de novo* to meet the intracellular demands (7). Thus, contrary to healthy cells, leukemic cells are more dependent on serum asparagine and glutamine to maintain cellular function. The unavailability of these amino acids causes disruption in cell metabolism, through faulty protein, DNA and RNA synthesis, resulting in apoptosis (5).

Asparaginase allergy

ASNase is an enzyme from bacterial origin, produced by either *Escherichia coli* (either native or with addition of polyethylene glycol (PEG) group) or *Erwinia chrysanthemi*. Origin of the drug influences its properties in different aspects. Because ASNase is a foreign protein to the body, it can illicit an immunogenic response. Immunogenicity may or may not be presented through symptoms (e.g., fever,

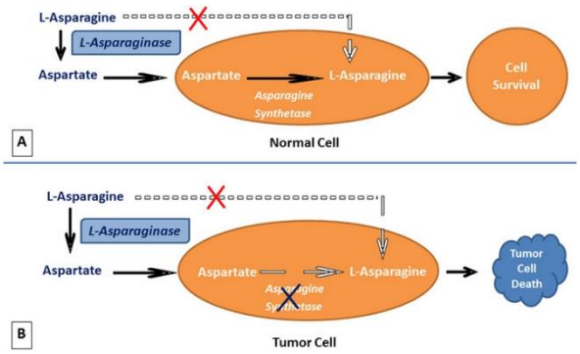

Figure 1: *L-asparaginase (ASNase) mechanism of action. ASNase catalyses the breakdown of serum asparagine. Healthy cells (A) can synthesize sufficient asparagine intracellularly due to the presence of asparagine synthase. Since expression of this enzyme is decreased in leukemic cells (B), asparagine is not available. Depletion of serum asparagine thus leads to cancer cell death. Figure adapted from Chand et al. (8).*

localized erythema, oedema, anaphylaxis, etc.) (10). Whereas the ASNase protein is the major antigen in native *E. coli* ASNase, it is the PEG-moiety targeted by antibodies in PEG-ASNase (9). Development of hypersensitivity (also called allergy when symptoms are present (11)) is the most frequent cause of ASNase discontinuation (10,12,13). Less common toxicities include pancreatitis, hyperglycaemia, and thrombosis. Allergy incidence varies with each type of ASNase formulation, being highest (10-30%) in native *E. coli* derived ASNase (10). According to a recent meta-analysis, risk factors for PEG-ASNase hypersensitivity are postinduction phase, a higher number of PEG-ASNase free intervals, and initiation of PEG-ASNase during postinduction phase (14). The influence of ASNase administration route on allergy is unclear, different studies show conflicting results when comparing intramuscular (IM) to intravenous (IV) administration (14-16).

ASNase hypersensitivity reactions can be categorized into three groups: 1) infusion-related complications, neutralizing antibodies are absent; 2) clinical allergy, either localized or generalized with corresponding symptoms, neutralizing antibodies are present; 3) silent inactivation, no presentation of clinical allergies, but neutralizing antibodies are present. Different approaches are required for each type of hypersensitivity reaction. While infusion-related problems can be solved relatively easily by decreasing ASNase infusion rate and by administration of pre-medication (e.g., antihistamines, acetaminophen, corticosteroids), severe allergic reactions or antibody formation are cause for ASNase discontinuation. Therapeutic drug monitoring provides insight to serum ASNase activity, thus playing an important role in determining treatment efficacy (5,17). Measuring ASNase activity is more feasible and reliable than the measurement of ASNase antibodies or ASNase concentrations.

Introduction of PEG-ASNase has been shown to be clinically relevant. Compared to the native *E. coli* ASNase, PEG-ASNase has a longer half-life, less antibody formation, and a lower allergy occurrence, making it the first treatment choice in most developed countries (12,14,17). Because there is effectively no cross-reactivity between *E. coli* derived ASNase and *E. chrysanthemi* derived ASNase, the latter formulation is used when allergies or antibody inactivation limit the use of *E. coli* derived ASNase. Note that because of pharmacokinetic differences, a different treatment schedule is maintained for *E. chrysanthemi* ASNase.

Initiating administration of *E. chrysanthemi* ASNase after *E. coli* ASNase discontinuation has been shown not to affect ASNase activity levels or event free survival (18-20). However, not receiving all prescribed ASNase dosages (either *E. coli* or *E. chrysanthemi* ASNase) did lead to a lower five-year event free survival rate (73%±7% vs 90%±2%; $p<0.01$) (21). A Children's Oncology Group analysis including more than 8,000 B-cell ALL patients also reported substantially lower event free survival rates in patients who did not receive all prescribed ASNase dosages (either PEG-ASNase or *E. chrysanthemi* ASNase), compared to those who did (20). However, this was seen only in standard risk patients who were on an intensified ASNase treatment protocol and in high risk patients. Interestingly, another study reported inferior outcomes only in ALL patients who received less than 50% of prescribed native *E. coli* ASNase dosages, hypothesizing that additional treatment with *E. chrysanthemi* ASNase might be unnecessary (22).

Asparaginase allergy and pharmacogenomics

Pharmacogenomics (also called pharmacogenetics) is the study that combines pharmacology and genetics, determining how patients' genetic profile can affect different aspects of a drug (e.g., pharmacokinetics and pharmacodynamics or efficacy and toxicity). Use of pharmacogenomics can contribute to medicine personalization by providing an underlying cause to interpatient variabilities to drugs and treatment success. For ASNase, different polymorphisms have been identified that can increase risk of allergy, thrombosis, and pancreatitis (23).

The human leucocyte antigen (HLA), also known as major histocompatibility complex (MHC) in humans, is a gene complex on chromosome six encoding proteins that play a pivotal role in immunologic responses. Class II HLA molecules (HLA-DP, -DQ, and -DR) are present on all antigen presenting cells and are considered to be the bridge between the innate and adaptive branch of the immune system. In the past, drug allergies have been linked to specific HLA alleles (e.g., allopurinol and carbamazepine severe cutaneous adverse reactions with HLA-DRB*5801 and HLA-DRB*1502, respectively) (24). Fernandez et al. were first to demonstrate that the HLA-DRB1*07:01 allele variant increased the risk of ASNase antibody formation and allergy. This was first observed in a candidate gene study and later established in a genome-wide association study (GWAS) (25,26). Subsequentially, HLA-DQA1*02:01 and HLA-DQB1*02:02, have also been associated with ASNase allergy in Caucasian patients, the DQA1*02:01 allele being in complete linkage disequilibrium[1] (LD) with DRB1*07:01 (27,28). Patients carrying all three HLA alleles have the highest risk for developing ASNase hypersensitivity, the occurrence hereof relying on the presence of HLA-DQB1*02:02, an allele which may be present amongst HLA-DRB1*07:01 carriers (28).

Aim

Currently, only a small study has evaluated the direct association between PEG-ASNase allergy and the presence of HLA alleles DRB1*07:01, DQA1*02:01 and DQB1*02:02 (29). This project aims to further investigate the relationship between these alleles and the clinical outcome allergy in a larger cohort of children with ALL treated according to the Dana-Farber Cancer Institute ALL Consortium Protocol 16-001 (DFCI 16-001). Additionally, a new DNA extraction method will be tested to reach this aim. Insight in the relationship between HLA allele variants and ASNase allergy can contribute to prediction of patients at risk of ASNase allergy development. As a result, adequate precautions can be taken prior to starting ASNase during ALL treatment and further improve clinical outcome.

Study population and methods

Patients

Children and adolescents aged between one and 22 years with newly diagnosed ALL were enrolled in the DFCI 16-001 study; a phase three, multicentre, open-label, randomized clinical trial with the primary objective to develop a new risk group categories to reduce toxicity but maintain rates of remission and overall event free survival. Patients received 2500 IU/m^2 IV PEG-ASNase (Oncaspar®) during induction and continued with either 2500 IU/m^2 (standard dose arm) or 2000 IU/m^2 (reduced dose arm) following randomization on a two-weekly schedule for 30 weeks. Dosage in the reduced dose arm was adjusted according to nadir serum ASNase activity (NSAA). If NSAA<0.4 IU/mL, the ASNase dosage was increased to 2500 IU/m^2. Similarly, if NSAA ≥1.0 IU/mL, the ASNase dosage was decreased to 1750 IU/m^2. PEG-ASNase allergy was assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. In the case of an allergic reaction grade three or higher, the patient was switched to *E. chrysanthemi* ASNase.

Patients included in the current analysis had provided consent through DFCI for future research and had either serum samples or DNA available. Patients excluded from current analysis were those who ceased ASNase treatment due to the development of pancreatitis or thrombosis or those who were taken off the current ALL protocol (e.g., due to presence of the Philadelphia chromosome etc.).

DNA extraction

DNA was extracted from serum samples using the QIAamp® Circulating Nucleic Acid Kit (QIAGEN GmbH, Hilden, Germany). A total of 10 mL of serum was used for extraction. In cases where less serum was available, patient serum was diluted with Dulbecco's phosphate-buffered saline (Life Technologies, USA) to reach a minimum amount of 4 mL necessary for extraction. Briefly, extraction consisted of cell lysis, binding of nucleic acids, washing of contaminants, and elution of nucleic acids. In cases where the total amount of DNA obtained was less than 200 ng, a whole genome amplification (WGA) step followed.

Whole genome amplification

Prior to WGA, DNA was blunted and ligated, as previously described (30,31). Blunting consisted of incubating 10 ng of extracted DNA for 20 minutes at 11 °C with 1 U T4 DNA polymerase, 5X reaction buffer (both purchased from Thermo Fisher Scientific,

[1]Linkage disequilibrium: the nonrandom association between alleles on loci in close physical proximity.

Carlsbad, CA, USA) and 2.5 mM dNTPs, total volume 20 µL. After incubation, the polymerase was inactivated for 10 minutes at 75 °C and samples were purified using MinElute® kit (QIAGEN GmbH, Hilden, Germany). All blunted DNA obtained was then ligated using 1 U T4 DNA ligase and 5X ligase buffer (both purchased from Invitrogen, Waltham, MA, USA). The total volume of 20 µL was incubated for 24 hours at 14 °C and purified using the MinElute® kit. The WGA protocol contained two steps. Firstly, primer annealing occurred. For this, 20 ng of ligated DNA was added to random primers (6-mers with the modification to prevent self-priming and non-specific amplification) and 10X PCR buffer. The total volume of 10 µL was incubated for 3 minutes at 95 °C, followed by 5 minutes at 4 °C for inactivation. Secondly, DNA was amplified using 100 U Phi29 DNA polymerase, 10X Phi29 buffer (both purchased from Lucigen, Middleton, WI, USA) and 2.5 mM dNTPs. The total volume of 10 µL was incubated for 20 hours at 30 °C followed by 20 minutes at 65 °C for inactivation.

Genotyping

After obtaining purified DNA, polymerase chain reaction (PCR) was used to multiply and tag DNA containing HLA-DRB1*07:01 and HLA-DQB1*02:02 haplotypes. Since HLA-DQA1*07:01 and HLA-DRB1*02:01 are in LD, the latter allele was not directly identified. Moving forward, HLA-DRB1*02:01 will not be mentioned. Recognition of the HLA-DRB1*07:01 allele was based on identification of SNP rs28724121 utilizing GTCCCCCAGACCCCCGTCCGCT and GGTCTCCGGGAAAAACACTG as forward and reverse primers respectively. Furthermore, for patients carrying the HLA-DRB1*07:01 allele, an additional two-step PCR was carried out to determine the presence of HLA-DQB1*02:02 based on SNP rs281863414. The first step of this two-step PCR is specific to two alleles and determines presence of either HLA-DQB1*02:02 or HLA-DQB1*02:01 utilizing TCCCTAGTCTGACCCCAGTG and AAATGGAACTGAAATAGCAAAAACG as forward and reverse primers respectively. The second step of this two-step PCR is more specific for HLA-DQB1*02:01 allele utilizing AAATTCATGATTAATCAATCC and AAATGGAACTGAAATAGCAACAACT as forward and reverse primers respectively. In case no amplification was detected in the second step of the two-step PCR, presence of HLA-DQB1*02:01 was excluded, establishing the presence of HLA-DQB1*02:02 only.

Analysis

Association between HLA allele variants and presence of allergy in this cohort was determined by univariate analyses. Significance was assumed when $p \leq 0.05$ and was determined using the Fisher exact test or the Chi-square test, accordingly. IBM SPSS Statistics version 27.0 (IBM, Chicago, IL, USA) was used to perform statistical analyses.

Results

DNA was extracted from 201 patients and total quantities obtained ranged between 40.05 and 3582 ng (mean and median 406.5 and 393.3 ng, respectively). WGA was performed on 33 samples. For 45 patients, DNA was readily available through the local biobank. While clinical data on allergy was available for 171 patients, 31 patients were excluded from this analysis, meaning that the final analysis consisted of 140 patients.

Of these patients, 24.3% experienced an allergic reaction grade two through four (table 1). The majority of the observed allergy cases (58.8%) being grade two. In 55.9% of the allergy cases, allergic reactions occurred before administration of the second PEG-ASNase dose.

Table 1: *allergy characteristics*

Allergy characteristic	**Number of patients (%)**
Allergy	
Present	34 (24.3%)
Absent	106 (75.7%)
Allergy grade	
Two	20 (58.8%)
Three	13 (38.2%)
Four	1 (2.9%)
Moment of allergy occurrence	
After dose one	19 (55.9%)
After dose two	12 (35.3%)
After dose three	3 (8.8%)

Amongst patients carrying the DRB1*07:01 allele, 25.7% experienced an allergic reaction (figure 2). However, no significant difference was found between allergy incidence in DRB1*07:01 carriers and DRB1*07:01 non-carriers (OR=1.1; 95% CI [0.5-2.7]; p=0.82). An additional analysis was done to determine the presence of DQB1*02:02 in patients carrying DRB1*07:01 (figure 3). Similarly, no significant

difference in allergy was found between patients harbouring both DRB1*07:01 and DQB1*02:02 (OR=1.4; 95% CI [0.4-5.0]; p=0.52).

To further analyse the effect of the DRB1*07:01/DQB1*02:02 haplotype, a stratification was made between low (grade two or less) and high (grade three or more) grade allergies. Patients carrying DRB1*07:01 and DQB1*02:02 initially appeared to be at a higher risk for developing high grade allergies compared to patients that were not positive for this haplotype (OR=2.9; 95% CI [0.7-11.9]). However, significance could not establish this (p=0.15).

Discussion

In this study, the relationship between PEG-ASNase allergy and HLA alleles DRB1*07:01 and DQB1*02:02 was determined. Since it is expected that PEG-ASNase will become the most common type of ASNase in ALL protocols, it is necessary to describe this association for this specific formulation. The development of an immune response to ASNase can lead to neutralizing antibody formation, inactivation of ASNase and, in worst cases, leukaemia relapse. Patients harbouring both DRB1*07:01 and

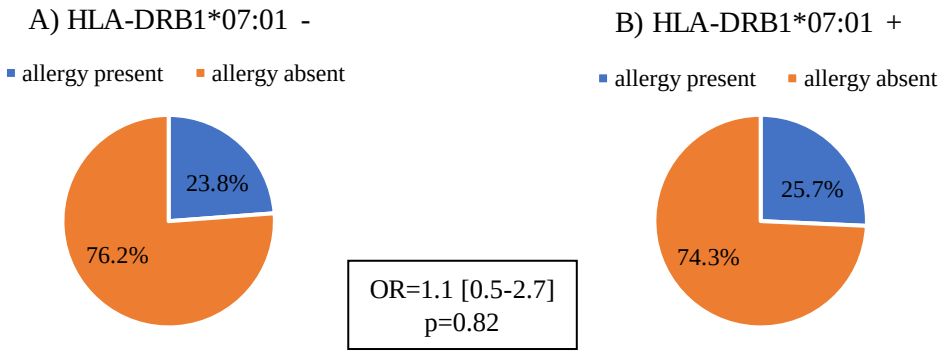

Figure 2: *Distribution of HLA-DRB1*07:01 and PEG-ASNase allergy. The incidence of allergy was 23.8% (25/105) in the HLA-DRB1*07:01 non-carriers (-) group (A), compared to 25.7% (9/35) in the HLA-DRB1*07:01 carriers (+) group (B). Odds Ratio (OR)= 1.1; 95% confidence interval [0.5-2.7]; p=0.82.*

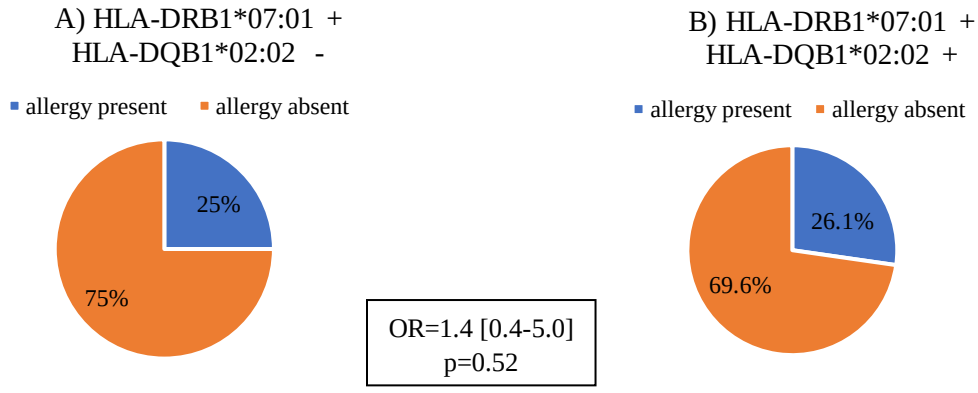

Figure 3: *Distribution of HLA-DRB1*07:01 and HLA-DQB1*02:02 combinations and PEG-ASNase allergy. The incidence of allergy was 25% (1/4) in the HLA-DRB1*07:01 carriers (+), HLA-DQB1*02:02 non-carriers (-) combination group (A), compared to 26.1% (6/23) in HLA-DRB1*07:01 carriers (+), HLA-DQB1*02:02 carriers (+) combination group (B). Odds Ratio (OR)= 1.4; 95% confidence interval [0.4-5.0]; p=0.52.*

DQB1*02:02 were expected to have the highest PEG-ASNase allergy incidence. However, current results are not in line with previous findings. For the single allele variation DRB1*07:01 and ASNase allergy, larger previous studies found OR between 1.64 and 4.1 (26-29). Similar results were found for the DRB1*07:01/DQB1*02:02 allele combination (OR between 2.6 and 5) (27,29). Although the current results show OR>1 for both DRB1*07:01 and DRB1*07:01/DQB1*02:02, no level of statistical significance ($p>0.05$) has been reached. Based on the absence of statistical significance, no conclusion can be made regarding this association in the current study population. It should be noted that the previous studies only included patients of European ancestry, making direct comparisons with this heterogenous study population difficult. Another contrast is the use of different types of ASNase formulations. The current DFCI protocol utilizes PEG-ASNase whereas native *E. coli* ASNase was generally used in former ALL protocols. However, Kondyli et al. showed that a small cohort of Caucasian patients receiving PEG-ASNase positive for DRB1*07:01/DQB1*02:02 had a higher risk of developing allergies (OR=3.9; $p=0.04$) (29). This could not be proven for DRB1*07:01 ($p>0.05$).

Total allergy incidence in this cohort was in line with literature reports. Notably, when comparing HLA allele variations to the higher grades of the allergic reaction, a p-value approaching statistical significance was found, introducing an interesting new perspective worth further investigation. Despite the higher grade allergic reactions having a larger clinical impact (e.g., requiring urgent care or hospitalization), unexpectedly, no grade one allergic reactions (e.g., transient flushing or rash, fever <38 degrees Celsius) were reported by clinicians in this cohort. In case these allergic reactions did occur but went unreported, the previously mentioned result must be interpreted cautiously.

A major limitation of current study is the lack of available information on patient characteristics and the lack of detail in the clinical data. Besides important information such as gender, age at diagnosis and ALL phenotype, a relevant patient characteristic is ethnic background. It is known that the HLA genes are highly polymorphic with SNPs distributed inconsistently amongst different populations worldwide, even acting as genetic landmarks to determine an individual's ancestry (32). The GWAS study by Fernandez et al. on ASNase hypersensitivity included patients of multiple ethnicities (European, African, Hispanic, Asian, and other) and was able to report the highest frequency of the HLA-DRB1 rs17885382 allele in patients of European descent and the lowest frequency in the Asian population (0.125 versus 0.051) (25). Considering that HLA-DRB1 rs17885382 is in LD with HLA-DRB1*07:01, information on patient ethnic background is deemed necessary to further analyse and interpret the current results. A recent publication concluded that unlike in patients of European descent, not the DRB1*07:01/DQB1*02:02 haplotype but rather the rs9958628 variant in ARHGAP28 (an allele involved in immune regulation) had the strongest association with PEG-ASNase hypersensitivity in non-Europeans, further demonstrating the importance of taking ancestry into consideration when developing pharmacogenetic markers (33). An attempt was made to decrease the confounding factor ethnicity by limiting study population heterogenicity. This was done by including only patients treated in institutions in Québec, Canada during the analysis, assuming the majority of these patients have a Caucasian background. Nevertheless, this did not further benefit the sought association between ASNase allergy and the DRB1*07:01/DQB1*02:02 haplotype.

Furthermore, data on patient PEG-ASNase randomization and detailed information of the experienced allergic reactions was missing at time of this analysis. The clinical data received for this project only specified allergy grading and cases where PEG-ASNase was ceased. It did not specify the type of allergic reaction (i.e., systemic or localized) or more importantly, cases where patients were successfully switched to *E. chrysanthemi* ASNase.

DNA was either obtained from institution biobank or, in most cases, from leftover patients' serum samples that were initially collected for PEG-ASNase activity measurement. In cases where minimal DNA was obtained from patient samples, DNA was multiplied with modified WGA protocols that included a ligation step. Without ligation, WGA will likely be unsuccessful on low quantities of DNA due to the high amount of existing damage to the DNA backbone. Thus, both ligation and WGA are necessary to acquire sufficient DNA quantities for PCR analysis. Although the standard WGA protocol had been used frequently, inclusion of a ligation step had not yet been done often. The obtained DNA after ligation and WGA appeared to be of inferior quantity and quality than the DNA directly extracted from patients' serum, requiring multiple PCR repetitions. Notably, patients who experienced complications during PEG-ASNase treatment had less serum samples available, e.g., due to early cessation of this drug. Consequentially, DNA

samples from these patients would more often be amplified using WGA. It is therefore likely that genotyping difficulties did not occur equally between patients with and without allergies.

Conclusion

In conclusion, this study presents an initial attempt to determine the association between specific HLA genetic markers and PEG-ASNase allergy in a paediatric ALL cohort treated according to DFCI 16-001. However, due to the limitations of current patient data, no definite conclusion about the effect of HLA-DRB1*07:01 and HLA-DQB1*02:02 on PEG-ASNase allergy can be made. Besides expanding study population size, incorporation of additional patient information is required. Most importantly, a stratification needs to be made between Caucasian and non-Caucasian patients and standard and low PEG-ASNase dosage.

Summary points
• ASNase is a key component of ALL treatment. • Due to its bacterial origin, ASNase is able to illicit an immune response, resulting in antibody formation that may be accompanied by allergic reactions. • Different pharmacogenetic studies have previously attributed the combination of HLA alleles DRB1*07:01, DQA1*02:01 and DQB1*02:02 to increase the risk of developing ASNase allergy. • This study was unable to find a significant association ($p>0.05$) between ASNase allergy and HLA alleles. • It is acknowledged that there are limitations. Primarily the ethnic background of the study population is expected to have introduced bias.

References

(1) Malard F, Mohty M. Acute lymphoblastic leukaemia. Lancet 2020 -04-04;395(10230):1146-1162.

(2) Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014;64(2):83-103.

(3) Pui C, Mullighan CG, Evans WE, Relling MV. Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood 2012 -08-09;120(6):1165-1174.

(4) Lee SHR, Yang JJ. Pharmacogenomics in acute lymphoblastic leukemia. Best Practice & Research Clinical Haematology 2017;30(3):229-236.

(5) Asselin B, Rizzari C. Asparaginase pharmacokinetics and implications of therapeutic drug monitoring. Leukemia & Lymphoma 2015 -08;56(8):2273.

(6) Hijiya N, Van Der Sluis IM. Asparaginase-associated toxicity in children with acute lymphoblastic leukemia. Leukemia & Lymphoma 2015 -11-20;57(4):748.

(7) Chiu M, Taurino G, Bianchi MG, Kilberg MS, Bussolati O. Asparagine Synthetase in Cancer: Beyond Acute Lymphoblastic Leukemia. Front Oncol 2019;9:1480.

(8) Chand S, Mahajan RV, Prasad JP, Sahoo DK, Mihooliya KN, Dhar MS, et al. A comprehensive review on microbial l-asparaginase: Bioprocessing, characterization, and industrial applications. Biotechnology and Applied Biochemistry 2020 July 1,;67(4):619-647.

(9) Liu Y, Smith CA, Panetta JC, Yang W, Thompson LE, Counts JP, et al. Antibodies Predict Pegaspargase Allergic Reactions and Failure of Rechallenge. Journal of clinical oncology 2019 Aug 10,;37(23):2051-2061.

(10) Burke MJ, Zalewska-Szewczyk B. Hypersensitivity reactions to asparaginase therapy in acute lymphoblastic leukemia: immunology and clinical consequences. Future Oncology 2022;18(10):1285.

(11) Schmiegelow K, Attarbaschi A, Barzilai S, Escherich G, Frandsen TL, Halsey C, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol 2016 -06;17(6):e231-e239.

(12) Cecconello DK, Magalhães MRd, Werlang ICR, Lee MLdM, Michalowski MB, Daudt LE. Asparaginase: an old drug with new questions. Hematology, Transfusion and Cell Therapy 2020;42(3):275-282.

(13) Egler RA, Ahuja SP, Matloub Y. L-asparaginase in the treatment of patients with acute lymphoblastic leukemia. J Pharmacol Pharmacother 2016;7(2):62-71.

(14) Brigitha LJ, Fiocco M, Pieters R, Albertsen BK, Escherich G, Lopez-Lopez E, et al. Hypersensitivity to Pegylated E.coli asparaginase as first-line treatment in contemporary paediatric acute lymphoblastic leukaemia protocols: a meta-analysis of the Ponte di Legno Toxicity working group. Eur J Cancer 2022 -02;162:65-75.

(15) Hasan H, Shaikh OM, Rassekh SR, Howard AF, Goddard K. Comparison of hypersensitivity rates to intravenous and intramuscular PEG-asparaginase in children with acute lymphoblastic leukemia: A meta-analysis and systematic review. Pediatric Blood & Cancer 2017;64(1):81-88.

(16) Burke MJ, Devidas M, Maloney K, Angiolillo A, Schore R, Dunsmore K, et al. Severe pegaspargase hypersensitivity reaction rates (grade ≥3) with intravenous infusion vs. intramuscular injection: analysis of 54,280 doses administered to 16,534 patients on children's oncology group (COG) clinical trials. Leuk Lymphoma 2018 -07;59(7):1624-1633.

(17) Sluis IMvd, Vrooman LM, Pieters R, Baruchel A, Escherich G, Goulden N, et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. 1 2016 /03/01;101(3):279-285.

(18) Tong WH, Pieters R, Kaspers GJL, te Loo, D. Maroeska W. M., Bierings MB, van den Bos C, et al. A prospective study on drug monitoring of PEGasparaginase and Erwinia asparaginase and asparaginase antibodies in pediatric acute lymphoblastic leukemia. Blood 2014 -3-27;123(13):2026-2033.

(19) Woo MH, Hak LJ, Storm MC, Sandlund JT, Ribeiro RC, Rivera GK, et al. Hypersensitivity or Development of Antibodies to Asparaginase Does Not Impact Treatment Outcome of Childhood Acute Lymphoblastic Leukemia. 1532 Journal of Clinical Oncology 2000 18:7, 1525-.

(20) Gupta S, Wang C, Raetz EA, Schore R, Salzer WL, Larsen EC, et al. Impact of Asparaginase Discontinuation on Outcome in Childhood Acute Lymphoblastic Leukemia: A Report From the Children's Oncology Group. J Clin Oncol 2020 -06-10;38(17):1897-1905.

(21) Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood 2001 -03-01;97(5):1211-1218.

(22) Yen H, Chang W, Liu H, Yeh T, Hung G, Wu K, et al. Outcomes Following Discontinuation of E. coli l-Asparaginase Upon Severe Allergic Reactions in Children With Acute Lymphoblastic Leukemia. Pediatric Blood & Cancer 2016 /04/01;63(4):665-670.

(23) Abaji R, Gagné V, Xu CJ, Spinella J, Ceppi F, Laverdière C, et al. Whole-exome sequencing identified genetic risk factors for asparaginase-related complications in childhood ALL patients. Oncotarget 2017 Jul 4,;8(27):43752-43767.

(24) Cheng C, Su S, Chen C, Chen W, Deng S, Chung W. HLA Associations and Clinical Implications in T-Cell Mediated Drug Hypersensitivity Reactions: An Updated Review. J Immunol Res 2014;2014.

(25) Fernandez CA, Smith C, Yang W, Mullighan CG, Qu C, Larsen E, et al. Genome-wide analysis links NFATC2 with asparaginase hypersensitivity. Blood 2015 -7-2;126(1):69-75.

(26) Fernandez CA, Smith C, Yang W, Daté M, Bashford D, Larsen E, et al. HLA-DRB1*07:01 is associated with a higher risk of asparaginase allergies. Blood 2014 -8-21;124(8):1266-1276.

(27) Gagné V, St-Onge P, Beaulieu P, Laverdière C, Leclerc J, Tran TH, et al. HLA alleles associated with asparaginase hypersensitivity in childhood ALL: a report from the DFCI Consortium. Pharmacogenomics 2020 -06;21(8):541.

(28) Kutszegi N, Yang X, Gézsi A, Schermann G, Erdélyi DJ, Semsei ÁF, et al. HLA-DRB1*07:01–HLA-DQA1*02:01–HLA-DQB1*02:02 haplotype is associated with a high risk of asparaginase hypersensitivity in acute lymphoblastic leukemia. Haematologica 2017 -9;102(9):1578-1586.

(29) Kondyli M, Tremblay D, Rezgui A, Serfaty SA, Gagné V, Ribère M, et al. Human Leucocyte Antigen alleles associated with asparaginase hypersensitivity in childhood Acute Lymphoblastic Leukemia patients treated with Pegylated asparaginase within Dana Farber Cancer Institute treatment protocols. Leukemia research 2021 Oct;109:106650.

(30) Brukner I, Paquin B, Belouchi M, Labuda D, Krajinovic M. Self-priming arrest by modified random oligonucleotides facilitates the quality control of whole genome amplification. Anal Biochem 2005 -04-15;339(2):345-347.

(31) Brukner I, Labuda D, Krajinovic M. Phi29-based amplification of small genomes. Anal Biochem 2006 -07-01;354(1):154-156.

(32) Vina MAF, Hollenbach JA, Lyke KE, Sztein MB, Maiers M, Klitz W, et al. Tracking human migrations by the analysis of the distribution of HLA alleles, lineages and haplotypes in closed and open populations. Philos Trans R Soc Lond B Biol Sci 2012 -3-19;367(1590):820-829.

(33) Liu Y, Yang W, Smith C, Cheng C, Karol SE, Larsen EC, et al. Class II HLA Variants Associate with Risk of Pegaspargase Hypersensitivity. Clin Pharmacol Ther 2021 -9;110(3):794-802.