

ORIGINAL ARTICLE

Drug Allergy, Insect Sting Allergy, and Anaphylaxis



WILEY

Drug-specific T-cell responses in patients with liver injury following treatment with the BACE inhibitor atabecestat

Paul J. Thomson¹ | Laila Kafu¹ | Xiaoli Meng¹ | Jan Snoeys² | An De Bondt³ | Dries De Maeyer³ | Hans Wils³ | Laurent Leclercq² | Petra Vinken⁴ | Dean J. Naisbitt¹

¹MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK

²Drug Metabolism and Pharmacokinetics, Janssen R&D, Beerse, Belgium

³Discovery Sciences, Janssen R&D, Beerse, Belgium

⁴Non-Clinical Safety, Janssen R&D, Beerse, Belgium

Correspondence

Dean J. Naisbitt, MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, Sherrington Building, Ashton Street, The University of Liverpool, Liverpool L69 3GE, UK. Email: dnes@liverpool.ac.uk

Funding information

PJT is a post-doctoral researcher that received funds from Janssen R&D to support his studies. The project also received funding from the MRC Centre for Drug Safety Science (Grant number G0700654) and an MRC project grant (Grant number MR/R009635/1).

Abstract

Background: Atabecestat is an orally administered BACE inhibitor developed to treat Alzheimer's disease. Elevations in hepatic enzymes were detected in a number of in trial patients, which resulted in termination of the drug development programme. Immunohistochemical characterization of liver tissue from an index case of atabecestat-mediated liver injury revealed an infiltration of T-lymphocytes in areas of hepatocellular damage. This coupled with the fact that liver injury had a delayed onset suggests that the adaptive immune system may be involved in the pathogenesis. The aim of this study was to generate and characterize atabecestat(metabolite)-responsive T-cell clones from patients with liver injury.

Methods: Peripheral blood mononuclear cells were cultured with atabecestat and its metabolites (diaminotiazine [DIAT], N-acetyl DIAT & epoxide) and cloning was attempted in a number of patients. Atabecestat(metabolite)-responsive clones were analysed in terms of T-cell phenotype, function, pathways of T-cell activation and cross-reactivity with structurally related compounds.

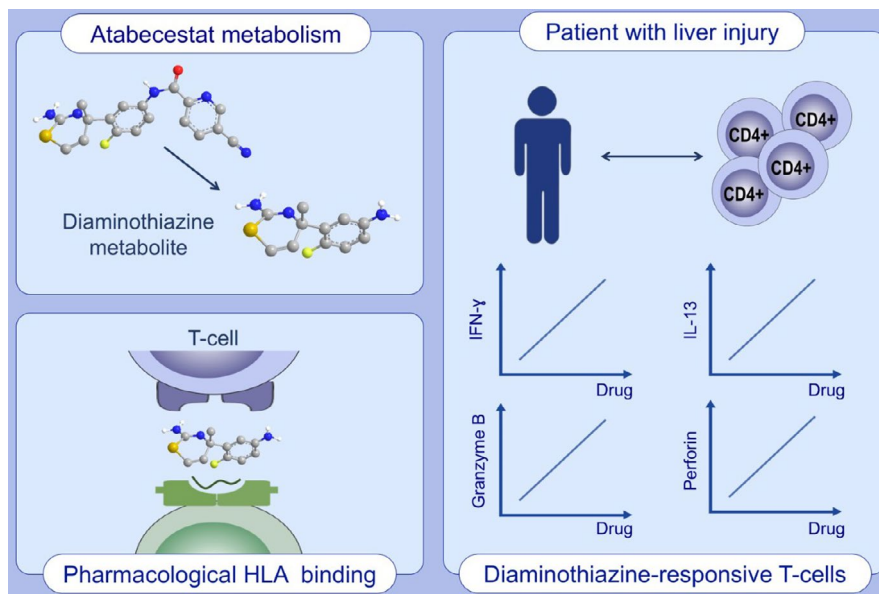
Results: CD4⁺ T-cell clones activated with the DIAT metabolite were detected in 5 out of 8 patients (up to 4.5% cloning efficiency). Lower numbers of CD4⁺ and CD8⁺ clones displayed reactivity against atabecestat. Clones proliferated and secreted IFN- γ , IL-13 and cytolytic molecules following atabecestat or DIAT stimulation. Certain atabecestat and DIAT-responsive clones cross-reacted with N-acetyl DIAT; however, no cross-reactivity was observed between atabecestat and DIAT. CD4⁺ clones were activated through a direct, reversible compound-HLA class II interaction with no requirement for protein processing.

Conclusion: The detection of atabecestat metabolite-responsive T-cell clones activated via a pharmacological interactions pathway in patients with liver injury is indicative of an immune-based mechanism for the observed hepatic enzyme elevations.

KEYWORDS

drug-induced liver injury, human, immune system, T-lymphocytes

Abbreviations: AD, Alzheimer's Disease; ALT, alanine transaminase; DILI, drug-induced liver injury; EBV, Epstein-Barr virus; HLA, human leucocyte antigen; PBMC, peripheral blood mononuclear cells; ULN, upper limit of normal.



GRAPHICAL ABSTRACT

The atabecestat diaminothiazine metabolite activates T-cells from patients with atabecestat-associated liver injury. CD4⁺ T-cell clones are activated through a direct, non-covalent drug (metabolite)-HLA class II interaction with no requirement for protein processing. It is likely that the adverse reactions experienced by these patients while on treatment with atabecestat were mediated at least in part by drug-responsive CD4⁺ T-cells.

1 | INTRODUCTION

Alzheimer's Disease (AD) is a chronic neurodegenerative disease which is characterized by an impairment in cognitive function beyond the scope of the normal ageing process. An imbalance exists between production and clearance of amyloid- β in patients with AD and a common disease characteristic is the presence of neurofibrillary tangles comprised of hyperphosphorylated tau proteins and amyloid plaques.¹ Amyloid- β is produced from amyloid precursor protein via a cleavage process by BACE-1 in the first and rate limiting step. Therefore, it is hypothesized that inhibition of the BACE-1 enzyme can reduce the formation of the toxic amyloid plaques, thereby having a positive impact on the progression of AD. Development of atabecestat (JNJ-54861911), an orally administered BACE-1 inhibitor, for the treatment of AD was recently stopped due to elevations in hepatic enzymes in clinical studies.²

Elevated hepatic enzymes were observed in patients participating in two atabecestat studies with 24% of patients exhibiting >1.5 upper limit of normal (ULN) levels of alanine transaminase (ALT), including 11% with ALT > 3x ULN. These were commonly associated with increases in aspartate aminotransferase (AST), but only 1 patient had a concomitant increase of bilirubin > 2x ULN in the presence of a normal alkaline phosphatase, indicative of severe hepatocellular injury. Importantly, increases in the level of ALT did not correlate with the concentration of atabecestat (metabolites) in patient plasma. A swift decline in ALT levels was observed in most patients following withdrawal of atabecestat; however, ALT levels continued to increase in the plasma of a limited number of patients. In particular, one patient reported an ALT level of 28.8x ULN one month after cessation of therapy. The delayed onset of liver injury and immuno-histological

detection of T-cell infiltrates in a liver biopsy specimen³ suggest that the adaptive immune system might be involved in the pathogenesis of the atabecestat-induced adverse event.

Until recently, the involvement of the adaptive immune system in drug-induced liver injury (DILI) was controversial. Maria and Victorino detected drug-responsive T-cells within peripheral blood mononuclear cells (PBMC) from certain patients with DILI.⁴ However, the drug-responsive T-cells were never characterized in terms of phenotype or function. More recently, we have shown the activation of drug-responsive T-cells from the blood of patients with flucloxacillin,⁵ amoxicillin-clavulanate⁶ and tuberculosis medication-induced liver injury.⁷ Moreover, several HLA allele associations have been identified in patients with DILI for a host of drugs including flucloxacillin (HLA-B*57:01),⁸ ximelagatran (HLA-DRB1*07:01)⁹ and lumiracoxib (HLA-DRB1:15:01)¹⁰ and in patients with drug rash with eosinophilia and systemic symptoms (DRESS; eg vancomycin¹¹ and dapsone¹²) where liver injury develops as a component of a multifaceted disease. Although not always proven through T-cell studies with patient PBMC, the genetic associations suggest that drugs interact preferentially with the protein (or binding peptide) derived from the HLA of interest to stimulate T-cells that contribute towards the disease pathogenesis. HLA molecules displayed on the cell surface present drugs bound directly via a reversible pharmacological interaction (p-I concept) and covalently bound drug peptide adducts generated through protein processing (hapten concept).

The aim of this study was to elucidate whether atabecestat-responsive T-cells were detectable in atabecestat trial patients that developed elevations in liver enzymes and then generate T-cell clones

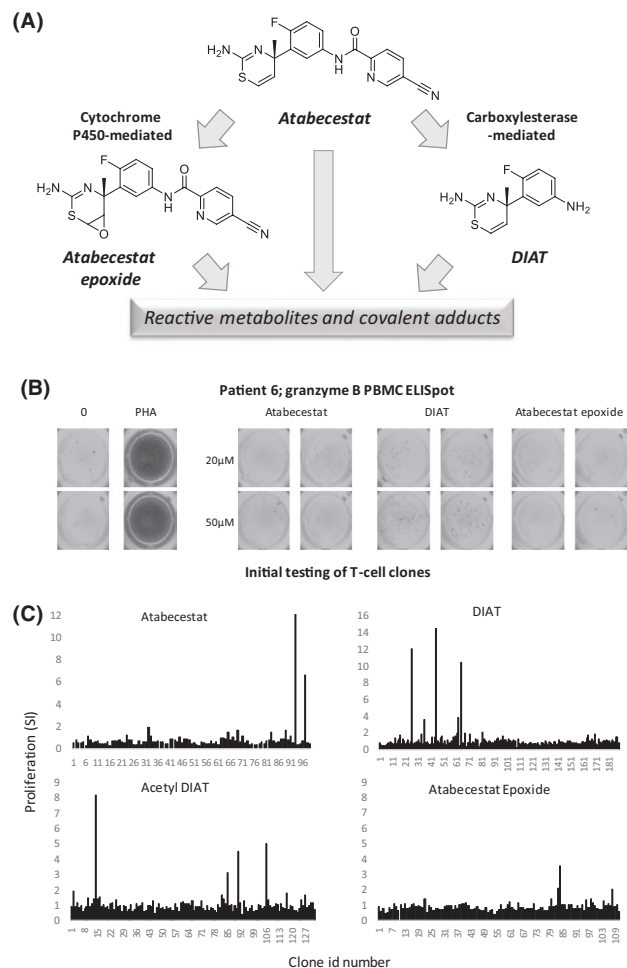


FIGURE 1 Study compound structures, granzyme B PBMC ELISpot and initial testing of T-cell clones from patient 6. A) Structure of atabecestat and its metabolic conversion to DIAT and atabecestat epoxide. B) PBMCs were incubated in a granzyme BELISpot plate with atabecestat, DIAT, acetyl DIAT and atabecestat epoxide for a period of 48 h. The ELISpot plate was developed and counted using ELISpot AID reader. C) T-cell clones from atabecestat-, DIAT-, acetyl DIAT- and atabecestat epoxide-derived T-cell lines (ie each figure panel shows separate clones) were co-incubated with autologous antigen-presenting cells in the presence and absence of atabecestat (10 $\mu\text{mol/L}$), DIAT (25 $\mu\text{mol/L}$), acetyl DIAT (25 $\mu\text{mol/L}$) and atabecestat epoxide (10 $\mu\text{mol/L}$). Cells were incubated for 48 h. [^3H] thymidine was then added for an additional 16-h incubation and T-cell proliferation was assessed using scintillation counting. Clones with a stimulation index of 1.5 or greater were selected and expanded for further analysis. Cross-reactivity was not possible at this early phase of the analysis

to characterize phenotype and function of the drug-responsive cells along with pathways of T-cell activation and cross-reactivity with related structures. Patient PBMC were cultured with synthetic forms of atabecestat, diaminothiazine [DIAT] and atabecestat epoxide, each of which are susceptible to cytochrome P450-mediated metabolism liberating products with the potential to bind covalently to protein (Figure 1A). DIAT is formed from atabecestat via the carboxylesterase-mediated hydrolysis of the amide bond resulting in loss of the cyanopyridine ring structure. In contrast, the epoxide of

atabecestat is formed on the amino-methyl-thiazinyl ring through cytochrome P450-3A mediated metabolism.

2 | METHODS

2.1 | PBMC from human subjects

Peripheral blood mononuclear cells were obtained from 14 study patients previously exposed to atabecestat (clinical studies 54861911ALZ2002, 54861911ALZ2003 and 54861911ALZ2004), of which 12 out of 14 had experienced liver ALT and AST elevations during therapy. The ALT increase in the selected patients ranged between 3.8 and 29.1x ULN. Table 1 summarizes the demographics of the patients and details of the adverse event. None of the patients showed skin symptoms or eosinophilia. PBMC from two healthy volunteers outside of the atabecestat studies were selected from the Liverpool cohort of 1200 genotyped healthy donors.¹³ Approval for the investigations was obtained from the Liverpool Research Ethics committee and each site's respective Ethic Committee, and informed written consent was obtained from each donor. Chemagic magnetic separation (Chemagen, Baesweiler, Germany) was used to extract genomic DNA, and high-resolution sequence-based HLA typing was performed by the Histogenetics laboratory (Histogenetics, Ossining, NY) at the following loci: HLA-A, -B, -C, -DRB1, -DQB1 and DQA1. PBMC isolated from blood using density centrifugation were cryopreserved on the day of isolation and stored in liquid nitrogen prior to conducting the experiments. Table S1 shows results of HLA typing.

PBMC were subjected to in vitro diagnostic testing and cloning of individual T-cells before characterization of cellular phenotype, TCR sequencing, function, antigen specificity and pathways of activation. Detailed methods are available in as Supplementary Material.

3 | RESULTS

3.1 | Atabecestat and its metabolites stimulate low levels of proliferation and granzyme B secretion in certain patients with liver injury

The lymphocyte proliferation assay was conducted in 10 patient PBMCs while ELISpot for IFN- γ and granzyme B was conducted on 9/14 patients (this included one patient not presenting ALT elevations; Table 1) to assess the presence of drug-responsive circulating T-cells in patients with liver injury. PBMCs from four subjects were not viable upon defrosting of vials, while cellular yield was low in 1 subject, excluding them from ELISpot assays.

Low levels of proliferation (stimulation index [proliferation in compound treated wells/ proliferation in medium wells] 2-2.5) were induced by the DIAT and epoxide metabolites in patient 4; however,

TABLE 1 Patient demographics and details of the adverse events

Patient id	Gender	Age	Country of origin	First study day ALT > 3xULN	Maximum ALT increase (xULN)	Dose at time of reaction	Time to study blood sampling following 1st ALT > 3X ULN	PBMC proliferation assay	PBMC ELISpot	
									IFN- γ	Granzyme B
Patient 1	F	71	Belgium	D 260	4.9	10 mg	758	-ve ^a	-ve	-ve
Patient 2	F	66	Belgium	/	/	5 mg	/	-ve	-ve	-ve
Patient 3	M	68	Belgium	D 168	4.5	10 mg	1024	-ve	n/a ^b	n/a
Patient 4	F	75	France	D 155	8.4	5 mg	468	-ve	-ve	-ve
Patient 5	M	63	Sweden	D 288	7.5	50 mg	691	-ve	-ve	-ve
Patient 6	F	76	UK	D 32	28.8	5 mg	544	-ve	-ve	+ve
Patient 7	M	73	Spain	D 344	6.5	10 mg	750	-ve	-ve	-ve
Patient 8	M	75	Belgium	/	/	50 mg	/	n/a	n/a	n/a
Patient 9	M	57	Germany	D 84	15.7	50 mg	975	-ve	-ve	-ve
Patient 10	F	68	France	D 260	9.4	25 mg	377	n/a	n/a	n/a
Patient 11	F	67	Australia	D 58	29.1	25 mg	306	n/a	n/a	n/a
Patient 12	F	64	UK	D 115	8.6	25 mg	152	-ve	-ve	-ve
Patient 13	F	67	UK	D 150	4.6	5 mg	141	-ve	-ve	-ve
Patient 14	F	76	USA	D 84	3.8	25 mg	84	n/a	n/a	n/a

Abbreviation: /, not applicable because no ALT elevation.

^aResult considered positive when stimulation index (response in drug treated/response in medium control) greater than 2.^bN/A – Assay not conducted due to low cell number/viability.

based on the low cpm values of the control condition and the size of the error bars, it is likely this was a false positive. All other subjects displayed no proliferation in the presence of any of the study compounds. Strong T-cell proliferation was induced with PBMC from all donors by the positive controls PHA and tetanus toxoid (data not shown).

Secretion of granzyme B was observed in response to the DIAT metabolite in patient 6, but no other compounds (Figure 1B). IFN- γ secretion was not detected. Furthermore, no secretion of IFN- γ or granzyme B was observed from PBMC of other patients with any of the study compounds. Strong secretion of IFN- γ and granzyme B was observed in response to PHA. Finally, no proliferation or secretion of IFN- γ or granzyme B was observed in healthy volunteers in response to atabecestat, DIAT and atabecestat epoxide.

3.2 | Atabecestat and its metabolites activate T-cell clones from patients with liver injury

T-cell clones were generated from patients with liver injury to assess cellular phenotype, drug cross-reactivity and pathways of drug presentation. During initial proliferation testing of clones with medium and study compounds, atabecestat (10 μ M), DIAT (25 μ M), acetyl DIAT (25 μ M) and atabecestat epoxide (10 μ M), using duplicate cultures, responsive T-cell clones were generated to atabecestat from patients 4, 5 and 6, while DIAT-responsive T-cell clones were generated from patients 1, 5, 6, 9 and 12 (Table S2). Acetyl DIAT-responsive clones were only generated from patient 6, while weakly-atabecestat epoxide-responsive clones were observed in patients 4, 6 and 12.

Confirmation of the responsiveness of T-cell clones after the first testing stage was via a dose-response study at compound concentrations of 0–100 μ M (duplicate conditions per culture condition). Four, ten and three T-cell clones were stimulated to proliferate in a dose-dependent manner with atabecestat, DIAT and acetyl DIAT, respectively (Figure S1A). The proliferative response of all clones generated from patient 6 is shown in Figure 1C. Treatment of the clones with atabecestat resulted in a reverse dose-response (lower proliferation at higher concentrations). This was not associated with atabecestat-induced T-cell toxicity; furthermore, concentrations of 10 μ M and below did not lead to significant levels of proliferation (results not shown). No responsive T-cell clones were identified to atabecestat epoxide. The total number of study compound-responsive T-cell clones and percentage of responsive clones generated during initial testing and after dose-responsive assessment from each patient is summarized in Table S2.

3.3 | Phenotype of study compound-responsive T-cell clones

With the exception of one CD8⁺ T-cell clone (patient 6; atabecestat-responsive) and one T-cell clone which was characterized as double positive (patient 5; DIAT-responsive), all responsive T-cell

clones to atabecestat, DIAT and acetyl DIAT were phenotyped as CD4⁺ (Figure S1B). Across the T-cell clones tested, a varied expression of TCR-V β was detected to the parent drug and the DIAT metabolite, with expression of rare TCRs (not detected by the antibodies in the kit) being predominantly expressed (Figure S1C). Chemokine receptor expression was assessed using six atabecestat- or DIAT-responsive clones expressing a single TCR. All tested T-cell clones expressed CXCR3, while the early activation marker CD69 and CCR4 were expressed on 5/6 clones. E-cadherin was expressed across some of the clones (Figure S1D). CXCR3 and CCR4 are involved in the recruitment of Th1 and Th2 cells,^{14,15} respectively.

3.4 | TCR sequencing

For sequencing analysis, all TCR α and TCR β CDR3 sequences with a fraction > 5% of the total reads were retrieved with a minimum total of >500 reads. Assuming these are indeed true single cell clones, we would expect only one functional TCR β CDR3 sequence in combination with maximum 2 functional TCR α CDR3 sequences for each T-cell clone. TCR sequencing performed on 6 atabecestat- or DIAT-responsive T-cell clones in total from 2 different patients revealed this was indeed the case for 4 clones (DIAT clones 1–3 and atabecestat clone 2). Two clones displaying 2 TCR β CDR3 sequences were detected suggesting that the expansion was not originating from a single clone. Nevertheless, the data from all 6 clones clearly show that atabecestat- and DIAT-responsive T-cells express different TCR β clonotypes and TCR α and β pairings. An overview of the T-cell clones that were sequenced is given in Table S3.

3.5 | Study compound-responsive T-cell clones exhibit distinct cytokine and cytolytic molecule secretion profiles

Secretion of cytokines and cytolytic molecules from one atabecestat- and five DIAT-responsive T-cell clones was assayed by ELISpot. Secretion of cytokines IFN- γ and IL-13 were detected from all clones. Three clones secreted IL-10 and IL-5, while low levels of IL-22 were also detected in three clones. IL-17 secretion was not detected. Secretion of granzyme B and perforin was detected following study compound treatment (Figure 2). Low levels of killing (2.4%–10.8%) of [⁵¹Cr]-loaded autologous antigen-presenting cells were observed with 3 study compound-treated clones (effector to target ratio 10:1; data not shown).

3.6 | Direct, reversible and HLA-DR-restricted activation of study compound-responsive CD4⁺ T-cell clones

To assess HLA dependency and class restriction of the CD4⁺ T-cell clone responses, HLA blocking studies were conducted. All CD4⁺

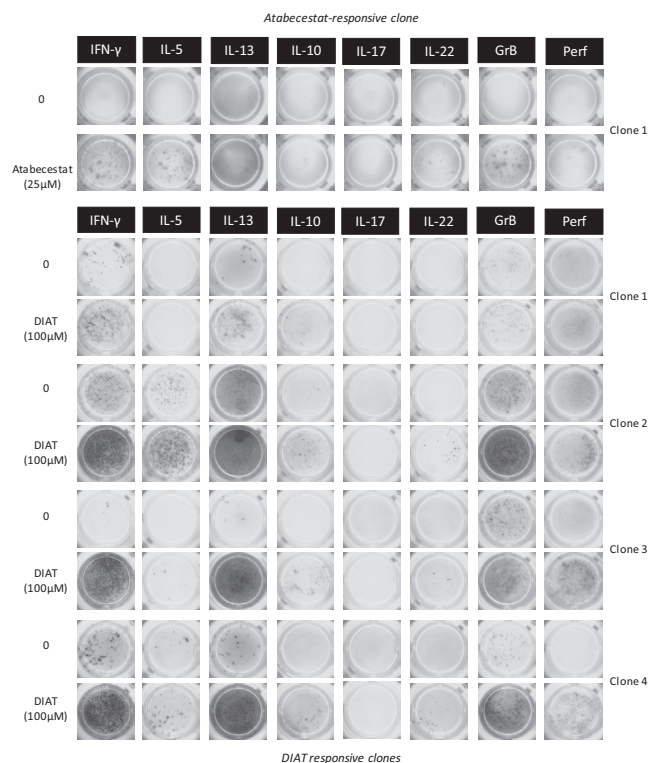


FIGURE 2 Analysis of cytokine and cytolytic molecule secretion from atabecestat- and DIAT-responsive T-cell clones. T-cell clones were co-incubated with autologous EBV-transformed antigen-presenting cells in the presence and absence of atabecestat (25 $\mu\text{mol/L}$) or DIAT (100 $\mu\text{mol/L}$) in an ELISPOT plate coated with antibodies for IFN- γ , granzyme B (GrB), IL-5, IL-13, IL-10, IL-17, IL-22 and perforin for 48 h. Following incubation, the ELISPOT plate was developed for the respective cytokines

clones responsive to atabecestat and DIAT demonstrated HLA-DR dependency for T-cell activation (Figure 3). The pathway of atabecestat- and DIAT-specific T-cell activation was assessed using antigen-presenting cell pulsing and glutaraldehyde fixation assays. T-cell clones exhibited no T-cell activation in the presence of antigen-presenting cells pulsed with study compounds for 1 or 16 hours. The small increase in proliferation observed with antigen-presenting cells pulsed with atabecestat for 1 hour was not significant ($P = .17$; Student's t test). Conversely, T-cell activity was observed, in a reduced capacity, to atabecestat and DIAT in the presence of glutaraldehyde-fixed antigen-presenting cells, but not in the absence of antigen-presenting cells (Figure 4).

3.7 | Study compound-responsive T-cell clones display clear patterns of cross-reactivity

The cross-reactivity profiles of T-cell clones were measured via co-incubation with EBV-transformed B cells and the four study compounds and assessment of proliferative responses or IFN- γ release. All T-cell clones generated to atabecestat yielded no cross-reactivity with DIAT, while 3/4 clones displayed proliferative responses with

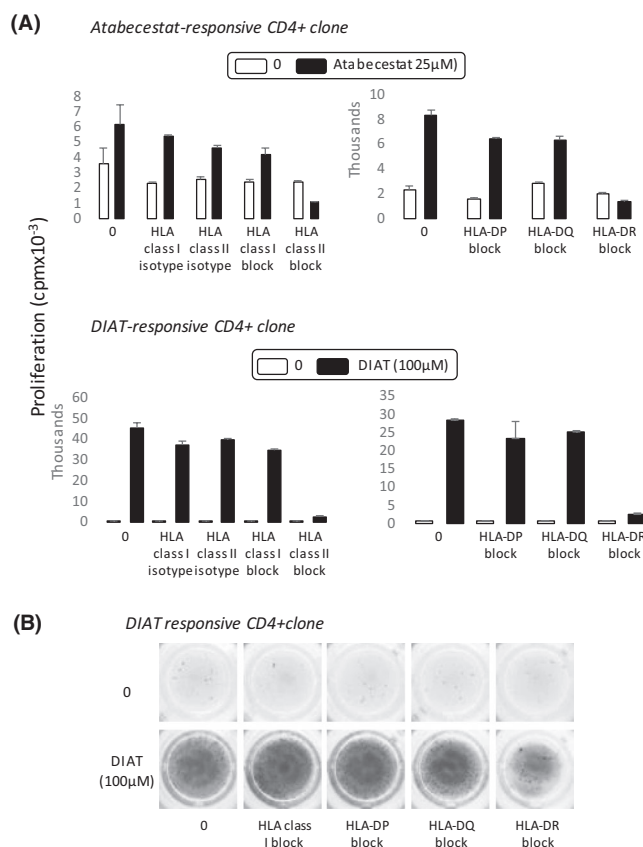


FIGURE 3 HLA-DR restriction of atabecestat and DIAT-responsive CD4⁺ T-cell clones. T-cell clones were co-incubated with autologous EBV-transformed antigen-presenting cells in the presence and absence of atabecestat (25 $\mu\text{mol/L}$) or DIAT (100 $\mu\text{mol/L}$) for 48 h. Prior to addition of the drugs, blocking antibodies; Isotype I, Isotype II, HLA Class I and HLA Class II were co-incubated with antigen-presenting cells and T-cell clones for 1 h. The assay was also conducted using HLA Class II sub-class-specific antibodies HLA-DP, DQ and DR. T-cell responses were quantified via analysis of (A) proliferation using [^3H] thymidine and scintillation counting or (B) IFN- γ secretion by way of ELISPOT

acetyl DIAT. A weak proliferative responses were also observed with atabecestat epoxide in one clone. DIAT-responsive T-cell clones exhibited no cross-reactivity with atabecestat or atabecestat epoxide. However, 3/6 DIAT-responsive T-cell clones from various patients exhibited cross-reactivity towards acetyl DIAT. Figure 5 shows representative clones displaying the different cross-reactivity profiles (atabecestat-specific, DIAT-specific and cross-reactive).

Figure 5 also shows the preferred energy minimized 3-dimensional spatial arrangement of atabecestat and its metabolites. The structures of atabecestat, DIAT and acetyl DIAT, with the exception of the side-chain moieties, were almost identical. However, the 3-dimensional spatial arrangement of atoms within atabecestat epoxide differed significantly due to the introduction of the 3-membered ring.

All three acetyl DIAT-responsive clones exhibited strong cross-reactivity towards the DIAT metabolite. One of these clones also cross-reacted with atabecestat (Figure S2).

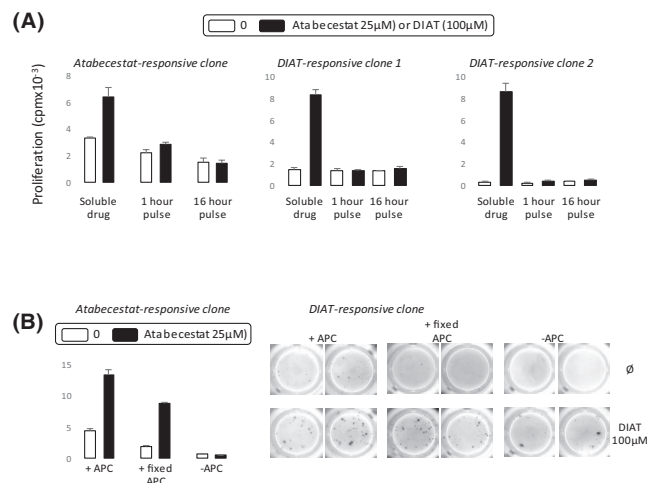


FIGURE 4 Direct activation of CD4⁺ T-cell clones with atabecestat and DIAT. A) T-cell clones were co-incubated with autologous EBV-transformed B-cells pre-pulsed with atabecestat (25 μmol/L) or DIAT (100 μmol/L) for 1 or 16 h. The study compound-pulsed EBV-transformed B cells were washed repeatedly to remove free compound prior to culturing with T-cells. B) T-cell clones were incubated with atabecestat (25 μmol/L) or DIAT (100 μmol/L) in the presence or absence of autologous EBV-transformed B cells (APC) fixed with glutaraldehyde to prevent protein processing. T-cell responses were measured either via analysis of proliferation through addition of [³H] thymidine and scintillation counting or through secretion of IFN-γ via ELISPOT

4 | DISCUSSION

Several forms of DILI are strongly associated with expression of one or a small number of HLA alleles,^{8-12,16-18} suggesting that a derivative of the drug may interact with a degree of selectivity with the HLA protein encoded by the HLA risk allele and a peptide embedded within the antigen-binding cleft of HLA to stimulate the T-cell response. Furthermore, drug-responsive T-cells have been detected in the circulation and inflamed liver of patients with DILI.^{5-7,19,20} Thus, it is becoming apparent that the adaptive immune system participates in the development of tissue pathology in genetically predisposed individuals. It must be emphasized that an HLA allele association only indicates a skewing in the preference for drug HLA peptide binding. There are many cases of immune-mediated DILI in patients that do not express HLA risk alleles. Furthermore, the majority of individuals that express HLA risk alleles do not go on to develop liver injury when exposed to culprit drugs. The best example of this is flucloxacillin-induced liver injury that preferentially develops in patients expressing HLA-B*57:01; however, the iatrogenic disease is only observed in 1 in 1000 HLA-B*57:01⁺ individuals exposed to the drug.⁸ All individuals are exposed to similar circulating concentrations of flucloxacillin and form the same protein adducts at levels that exceed the threshold for T-cell activation.^{5,21-25} Hence, additional factors must impact on susceptibility.

In this study we focused on the therapeutic atabecestat, which functioned to reduce the synthesis of amyloid plaques, a significant

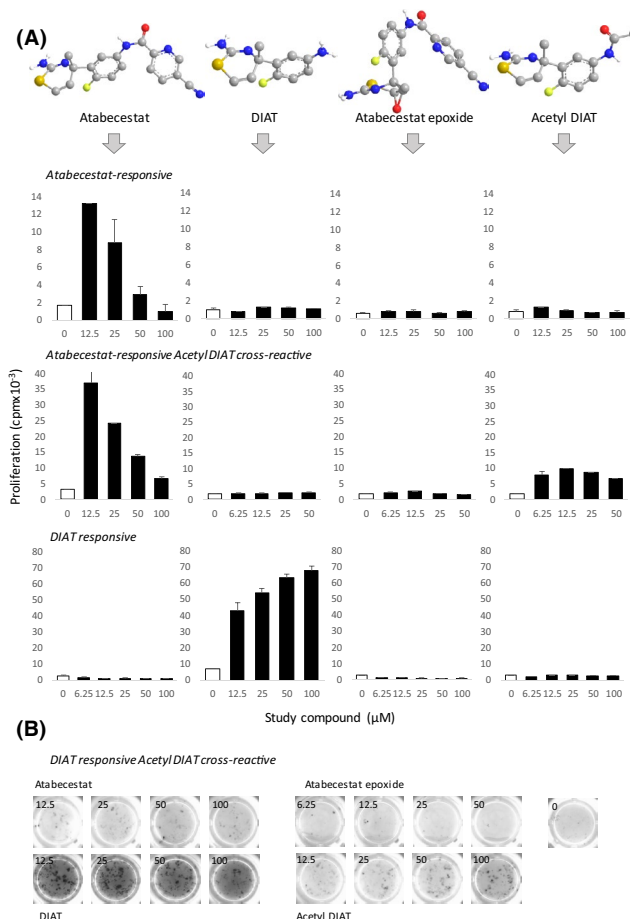


FIGURE 5 Cross-reactivity of study compound-specific T-cell clones. (A) Energy minimized 3D-structures of study compounds. Proliferation of representative T-cell clones in the presence of study compounds. T-cell clones were co-incubated with autologous EBV-transformed B cells in the presence and absence of atabecestat, DIAT, atabecestat epoxide and acetyl DIAT for 48 h. [³H] thymidine was added for an additional 16 h and proliferation was measured via scintillation counting. Error bars denote ± SEM. (B) IFN-γ secretion from study compound-stimulated T-cell clones. T-cell clones were co-incubated with autologous EBV-transformed B cells in the presence and absence of atabecestat, DIAT, atabecestat epoxide and acetyl DIAT in a ELISPOT plate for 48 h. Following incubation, the ELISPOT was developed for IFN-γ

feature of AD. Phase I studies were conducted to atabecestat in amyloid positive Caucasian and Japanese patients. Four-week treatment with atabecestat resulted in a mean reduction of 67% in cerebrospinal fluid-Aβ₁₋₄₀ in both the Caucasian and Japanese patients, with a reduction of up to 90% in some patients.²⁶ Following this, phase 2/3 studies were conducted, with a plan to enrol up to 1650 patients at risk of dementia with a primary endpoint being the slowing of cognitive decline in those patients receiving either 10 mg (decreased to 5 mg after 11 participants received 10 mg to increase safety margin between low and high doses) or 25 mg of the drug compared to placebo. However, in May 2018 the atabecestat programme was discontinued based on the detection of elevated liver enzymes. Of the

475 patients exposed to atabecestat, approximately 24% had ALT/AST of $>1.5 \times$ ULN, and 11% had ALT elevations $>3 \times$ ULN. The evolution of these elevations was heterogeneous and some elevations were transient while patients remained on treatment. The patients with liver injury showed no signs of skin rash and/or eosinophilia. Interestingly, the dose of atabecestat (10–25 mg) is relatively low for a DILI-causing drug and a genetic association with HLA has not been identified. Furthermore, the time to onset of raised liver enzymes (32–344 days; Table 1) is significantly longer than that associated with skin reactions such as maculopapular exanthema. Although the factors responsible for breaking tolerance after several months of drug therapy are not known, the expression and activity of immune co-inhibitory receptors such as PD-1 and CTLA-4 are an area of considerable interest. Through in vitro assessment of drug-specific T-cell priming, we have shown that the level of T-cell activation is significantly enhanced when immune regulation has been dysregulated with immune checkpoint inhibitors.^{27,28} Clinical studies using a low molecular weight drug alongside an immune checkpoint inhibitor are also beginning to highlight the importance of immune dysregulation on susceptibility to DILI. For example, dacarbazine, a relatively safe imidazole carboxamide chemotherapy drug, was not tolerable in a Japanese phase II study when combined with ipilimumab, a monoclonal antibody that targets CTLA-4. Twelve out of fifteen patients developed elevations in ALT and aspartate aminotransferase.²⁹ Hence, it is possible that a natural imbalance in immune regulatory factors mediated by disease or environmental factors may be an important determinant of susceptibility. Alternatively, a low drug-responsive T-cell precursor frequency at the time of initial drug exposure might explain the delayed time to onset, but not the variable time to onset.

To investigate the involvement of the immune system in subjects who experienced elevated liver enzymes while receiving atabecestat, PBMC samples were collected, and T-cells were cloned from atabecestat- and atabecestat metabolite-exposed PBMC lines for phenotypic and functional assessment. HLA-genotyping was conducted on four of the patients PBMC that were subjected to T-cell cloning. Genotyping identified the common expression of HLA-DRB1*04:01 and DRB1*15:01 in three patients suggesting that if CD4⁺ T-cell responses were detected they may involve the drug interacting selectively with the proteins encoded by these alleles.

On the whole, diagnostic assays conducted on the PBMCs of patients were negative by way of proliferation and secretion of cytokines and effector molecules. One exception was patient 6, which noted a subtle increase in secretion of granzyme B in response to the primary metabolite of atabecestat; DIAT. Patient 6 showed persistent liver enzyme elevations even one month after cessation of atabecestat treatment. Furthermore, a liver biopsy from this patient indicated a zone 3-predominant necroinflammatory process, with lymphocyte infiltration.³

Eight patient PBMC were subjected to T-cell cloning. Study compound-responsive clones were generated from five of these patients with the greatest number stemming from patient 6. Generation of atabecestat-responsive T-cell clones was much more uncommon than seen with the DIAT metabolite, which may suggest a lower

T-cell precursor frequency. Furthermore, no confirmed T-cell clones were generated to the minor epoxide metabolite of atabecestat suggesting that if atabecestat epoxide-responsive clones exist, their precursor frequency must be very low. In total, atabecestat-responsive clones were generated from two of the patients PBMC to undergo T-cell cloning. One of the atabecestat-responsive clones exhibited a CD8⁺ phenotype in contrast with all other generated clones which were CD4⁺. DIAT-responsive clones detected in 5 patients all displayed a CD4⁺ phenotype. Clones were activated with study compounds at concentrations of 10 μ mol/L and above. Steady-state human atabecestat plasma levels are reached by day 5 in line with the plasma $t_{1/2}$ of 15–19h. At 25 mg qd atabecestat dosing, a mean plasma C_{max} of 0.7 μ mol/L was observed.^{26,30} All circulating human metabolites showed a similar plasma half-life as atabecestat. The most abundant human plasma metabolite was the DIAT metabolite which reached a steady-state C_{max} of 0.05 μ mol/L. Although atabecestat drug-related material was not quantified in human liver samples, an atabecestat pharmacokinetic study was conducted in TK-NOG humanized chimeric liver mice showing 15-fold higher atabecestat drug-related material concentrations in humanized liver vs plasma. The DIAT metabolite concentrations were on average 12% of the atabecestat concentrations in liver extracts from this study. Collectively, this indicates that the concentration of atabecestat in plasma is lower than that needed to activate T-cells; however, T-cell stimulatory concentrations may be reached when the drug accumulates in liver. Atabecestat- and DIAT-responsive clones exhibited a varied expression of TCR-V β with the majority expressing a rare TCR not detected by the antibody repertoire. Given the differential reactivity profiles of the clones, it is unlikely that TCR expression is conserved across the clones. For example, two DIAT-responsive T-cell clones expressing rare TCR-V β s exhibited cross-reactivity towards other study compounds, while two additional clones from the same patient exhibited no cross-reactivity.

Stimulation of clones with atabecestat or DIAT resulted in the secretion of the Th1 cytokine IFN- γ and Th2 cytokines IL-5 and IL-13. Furthermore, IL-10 and IL-22 were secreted from a more restricted number of study compound-activated clones. Secretion of Th1 and Th2 cytokines, alongside IL-22, is a common feature for drug-responsive clones isolated from patients presenting with skin and liver conditions.^{6,21,31} IL-10 secretion is observed less frequently. IL-10 is predominantly immunoregulatory, ameliorating excessive and potentially pathogenic Th1 and CD8⁺ T-cell responses.³² The activated clones, which for the most part were CD4⁺, also secreted effector molecules perforin and granzyme B. These molecules act together, with perforin inserting pores into neighbouring cells to permit the delivery of granzyme B, which cleaves caspases triggering the apoptotic cascade.

All tested atabecestat- and DIAT-responsive CD4⁺ clones were activated in a manner restricted to a MHC Class II protein, namely HLA-DR. As discussed above, clones were generated from three patients that expressed the HLA-DR*B alleles 04:01 and 15:01. However, genotyping conducted on patient 6, the patient yielding the largest number of study compound-responsive clones and exhibited the most serious clinical symptoms, indicated the expression

of HLA-DR*B alleles 12:01 and 13:02. This suggests that T-cell responses to atabecestat and DIAT in subjects are not restricted to the expression of one single HLA allele. Future experiments are planned using antigen-presenting cells from donors expressing different HLA alleles to define the HLA-DR molecules involved in presentation of atabecestat and its metabolites to T-cells. With this knowledge, antigen-presenting cell pulsing experiments were conducted to characterize pathway(s) of atabecestat and DIAT-specific CD4⁺ T-cell activation. Antigen-presenting cells were pulsed with study compounds for 1 or 16 hours prior to repeated washing to remove soluble and weakly associated compound prior to culturing the antigen-presenting cells with T-cells. Antigen-presenting cells pulsed with protein-reactive compounds (eg flucloxacillin, nitroso sulphamethoxazole^{5,33,34}) or compounds that associate strongly with HLA (eg abacavir³⁵⁻³⁷) activate T-cell responses, while compounds that associate weakly with HLA via reversible binding interactions (eg carbamazepine^{34,38-40}) do not. All atabecestat- or DIAT-responsive clones were not activated with study compound-pulsed antigen-presenting cells. This indicates that the clones are stimulated with atabecestat and DIAT via a non-covalent pharmacological binding interaction with HLA-DR molecules. Activation of T-cells in the presence of glutaraldehyde-fixed antigen-presenting cells was also observed, which is indicative of a direct interaction of atabecestat and DIAT with HLA-DR molecules in a manner independent of antigen processing. Fixation blocks protein processing and inhibits T-cell responses to protein antigens, but not antigenic peptides that bind directly to surface HLA molecules.³⁸⁻⁴³ Collectively, these findings are in agreement with the p-I concept proposed by Pichler.⁴⁴ In essence, atabecestat and its metabolites have an undesirable (off-target) pharmacological interaction with immune receptors. The p-I concept also argues that certain drugs may bind initially to highly variable TCRs and that HLA proteins provide additional signals for T-cell activation. Identification of a drug-specific public TCR that drives severe carbamazepine-induced cutaneous reactions provides strong support for this concept.⁴⁵ TCR sequencing was performed using six atabecestat or DIAT-responsive clones from two patients to determine whether a single rare TCR was identifiable. Although our data set was somewhat limited, it clearly shows that the drug-responsive clones express different TCRβ clonotypes and TCRα and β pairings. Given the low circulating concentrations in human plasma and the absence of an association of liver injury with an HLA allele, atabecestat and its metabolites must bind with high affinity with multiple HLA proteins to activate the patient T-cells. Future research should attempt to determine whether there is a similar drug binding site which is common to the presenting HLA proteins.

Atabecestat- and DIAT-responsive clones displayed either no cross-reactivity or cross-reacted with acetyl DIAT (Figure 4). Based on these findings, clones were generated from PBMC cultured with acetyl DIAT for further assessment of cross-reactivity. Interestingly, acetyl DIAT-responsive clones displayed cross-reactivity with DIAT and in one clone atabecestat and DIAT (Figure S2). Since all of the study compounds activate the T-cells through a non-covalent pharmacological binding interaction with HLA, with no requirement for

protein processing, the similar spatial arrangement of atoms between atabecestat, DIAT and acetyl DIAT provides one explanation for the observed reactivity profiles of the clones. The acetyl-cyanopyridine structure that differentiates atabecestat from DIAT must provide an essential HLA peptide binding interaction for the activation of atabecestat-responsive clones, whereas with DIAT-responsive clones, sufficient binding energy must be provided by the remainder of the drug structure. Acetyl DIAT represents an intermediate structure, which explains its capacity to activate certain atabecestat- and DIAT-responsive clones. Very little reactivity was observed towards atabecestat epoxide, which exists in a very different confirmation (Figure 4).

We must consider the fact that these data are taken from a relatively small number of T-cell clones; nevertheless, our findings show that drug-responsive T-cells with specificity to atabecestat and its DIAT metabolite are present in patients with atabecestat-induced liver injury. Thus, it is likely that the DILI reactions experienced by these patients while on treatment with atabecestat were mediated at least in part by the adaptive immune system, with CD4⁺ responses being the primary action. Furthermore, it is probable that such reactions were mediated to a greater degree by the DIAT metabolite of atabecestat. Future experiments profiling gene expression in drug-responsive T-cell clones and T-cell infiltrating liver of the same patients will provide direct evidence that circulating atabecestat- and/or DIAT-responsive T-cells are responsible for the tissue injury.

ACKNOWLEDGMENTS

The authors would like to thank the patients and volunteers for their generous donations.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

PJT, LK and XM conducted the biological experiments. LL, PV and JS designed the clinical protocols and collected the patient samples. ADB, DDM and HW conducted the TCR sequencing experiments. DJN, JS and PV designed the research study and supervised the project. PJT, LK and DJN analysed the data and drafted the manuscript. All authors critically reviewed the manuscript.

ORCID

Dean J. Naisbitt  <https://orcid.org/0000-0003-4107-7832>

REFERENCES

- Sanabria-Castro A, Alvarado-Echeverria I, Monge-Bonilla C. Molecular pathogenesis of Alzheimer's disease: an update. *Ann Neurosci*. 2017;24(1):46-54.
- Henley D, Raghavan N, Sperling R, Aisen P, Raman R, Romano G. Preliminary results of a trial of atabecestat in preclinical Alzheimer's disease. *N Engl J Med*. 2019;380(15):1483-1485.
- De Jonghe S, Weinstock D, Aligo J, Washington K, Naisbitt D. Biopsy pathology and immunohistochemistry of a case of

- immune-mediated drug-induced liver injury with Atabecestat. *Hepatology*. 2020; <https://doi.org/10.1002/hep.31403>
4. Maria VA, Victorino RM. Diagnostic value of specific T cell reactivity to drugs in 95 cases of drug induced liver injury. *Gut*. 1997;41(4):534-540.
 5. Monshi MM, Faulkner L, Gibson A, et al. Human leukocyte antigen (HLA)-B*57:01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology*. 2013;57(2):727-739.
 6. Kim SH, Saide K, Farrell J, et al. Characterization of amoxicillin- and clavulanic acid-specific T cells in patients with amoxicillin-clavulanate-induced liver injury. *Hepatology*. 2015;62(3):887-899.
 7. Usui T, Meng X, Saide K, et al. From the Cover: Characterization of isoniazid-specific T-cell clones in patients with anti-tuberculosis drug-related liver and skin injury. *Toxicol Sci*. 2017;155(2):420-431.
 8. Daly AK, Donaldson PT, Bhatnagar P, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet*. 2009;41(7):816-819.
 9. Hirasawa M, Hagihara K, Abe K, Ando O, Hirayama N. In Silico and In Vitro Analysis of Interaction between Ximelagatran and Human Leukocyte Antigen (HLA)-DRB1*07:01. *Int J Mol Sci*. 2017;18(4):694.
 10. Singer JB, Lewitzky S, Leroy E, et al. A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet*. 2010;42(8):711-714.
 11. Konvinse KC, Trubiano JA, Pavlos R, et al. HLA-A*32:01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. *J Allergy Clin Immunol*. 2019;144(1):183-192.
 12. Zhang FR, Liu H, Irwanto A, et al. HLA-B*13:01 and the dapsone hypersensitivity syndrome. *N Engl J Med*. 2013;369(17):1620-1628.
 13. Faulkner L, Gibson A, Sullivan A, et al. Detection of primary T cell responses to drugs and chemicals in HLA-typed volunteers: implications for the prediction of drug immunogenicity. *Toxicol Sci*. 2016;154(2):416-429.
 14. Sebastiani S, Allavena P, Albanesi C, et al. Chemokine receptor expression and function in CD4⁺ T lymphocytes with regulatory activity. *J Immunol*. 2001;166(2):996-1002.
 15. Bonecchi R, Bianchi G, Bordignon PP, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med*. 1998;187(1):129-134.
 16. Nicoletti P, Aithal GP, Bjornsson ES, et al. Association of liver injury from specific drugs, or groups of drugs, with polymorphisms in HLA and other genes in a genome-wide association study. *Gastroenterology*. 2017;152(5):1078-1089.
 17. Lucena MI, Molokhia M, Shen Y, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. *Gastroenterology*. 2011;141(1):338-347.
 18. Urban TJ, Nicoletti P, Chalasani N, et al. Minocycline hepatotoxicity: clinical characterization and identification of HLA-B*35:02 as a risk factor. *J Hepatol*. 2017;67(1):137-144.
 19. Mennicke M, Zawodniak A, Keller M, et al. Fulminant liver failure after vancomycin in a sulfasalazine-induced DRESS syndrome: fatal recurrence after liver transplantation. *Am J Transplant*. 2009;9(9):2197-2202.
 20. Wullemijn N, Terracciano L, Beltraminelli H, et al. T cells infiltrate the liver and kill hepatocytes in HLA-B(*)57:01-associated flucloxacillin-induced liver injury. *Am J Pathol*. 2014;184(6):1677-1682.
 21. Meng X, Al-Attar Z, Yaseen FS, et al. Definition of the nature and hapten threshold of the beta-lactam antigen required for T cell activation in vitro and in patients. *J Immunol*. 2017;198(11):4217-4227.
 22. Jenkins RE, Meng X, Elliott VL, Kitteringham NR, Pirmohamed M, Park BK. Characterisation of flucloxacillin and 5-hydroxymethyl flucloxacillin haptenated HSA in vitro and in vivo. *Proteomics Clin Appl*. 2009;3(6):720-729.
 23. Bechara R, Maillere B, Joseph D, Weaver RJ, Pallardy M. Identification and characterization of a naive CD8⁺ T cell repertoire for benzylpenicillin. *Clin Exp Allergy*. 2019;49(5):636-643.
 24. Azoury ME, Fili L, Bechara R, et al. Identification of T-cell epitopes from benzylpenicillin conjugated to human serum albumin and implication in penicillin allergy. *Allergy*. 2018;73(8):1662-1672.
 25. Scornet N, Delarue-Cochin S, Azoury ME, et al. Bioinspired design and oriented synthesis of immunogenic site-specifically penicilloylated peptides. *Bioconjug Chem*. 2016;27(11):2629-2645.
 26. Timmers M, Streffer JR, Russu A, et al. Pharmacodynamics of atabecestat (JNJ-54861911), an oral BACE1 inhibitor in patients with early Alzheimer's disease: randomized, double-blind, placebo-controlled study. *Alzheimers Res Ther*. 2018;10(1):85.
 27. Gibson A, Faulkner L, Lichtenfels M, et al. The effect of inhibitory signals on the priming of drug hapten-specific T cells that express distinct vbeta receptors. *J Immunol*. 2017;199(4):1223-1237.
 28. Naisbitt DJ, Olsson-Brown A, Gibson A, et al. Immune dysregulation increases the incidence of delayed-type drug hypersensitivity reactions. *Allergy*. 2020;75(4):781-797.
 29. Yamazaki N, Uhara H, Fukushima S, et al. Phase II study of the immune-checkpoint inhibitor ipilimumab plus dacarbazine in Japanese patients with previously untreated, unresectable or metastatic melanoma. *Cancer Chemother Pharmacol*. 2015;76(5):969-975.
 30. Novak G, Streffer JR, Timmers M, et al. Long-term safety and tolerability of atabecestat (JNJ-54861911), an oral BACE1 inhibitor, in early Alzheimer's disease spectrum patients: a randomized, double-blind, placebo-controlled study and a two-period extension study. *Alzheimers Res Ther*. 2020;12:58.
 31. Zhao Q, Alhilali K, Alzahrani A, et al. Dapsone- and nitroso dapsone-specific activation of T cells from hypersensitive patients expressing the risk allele HLA-B*13:01. *Allergy*. 2019;74(8):1533-1548.
 32. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol*. 2008;180(9):5771-5777.
 33. Wullemijn N, Adam J, Fontana S, Krahenbuhl S, Pichler WJ, Yerly D. HLA haplotype determines hapten or p-i T cell reactivity to flucloxacillin. *J Immunol*. 2013;190(10):4956-4964.
 34. Castrejon JL, Berry N, El-Ghaiesh S, et al. Stimulation of human T cells with sulfonamides and sulfonamide metabolites. *J Allergy Clin Immunol*. 2010;125(2):411-418.
 35. Illing PT, Vivian JP, Dudek NL, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature*. 2012;486(7404):554-558.
 36. Ostrov DA, Grant BJ, Pompeu YA, et al. Drug hypersensitivity caused by alteration of the MHC-presented self-peptide repertoire. *Proc Natl Acad Sci USA*. 2012;109(25):9959-9964.
 37. Adam J, Eriksson KK, Schnyder B, Fontana S, Pichler WJ, Yerly D. Avidity determines T-cell reactivity in abacavir hypersensitivity. *Eur J Immunol*. 2012;42(7):1706-1716.
 38. Schnyder B, Burkhart C, Schnyder-Frutig K, et al. Recognition of sulfamethoxazole and its reactive metabolites by drug-specific CD4(+) T cells from allergic individuals. *J Immunol*. 2000;164(12):6647-6654.
 39. Wu Y, Sanderson JP, Farrell J, et al. Activation of T cells by carbamazepine and carbamazepine metabolites. *J Allergy Clin Immunol*. 2006;118(1):233-241.
 40. Burkhart C, von Greysen S, Depta JP, et al. Influence of reduced glutathione on the proliferative response of sulfamethoxazole-specific and sulfamethoxazole-metabolite-specific human CD4⁺ T-cells. *Br J Pharmacol*. 2001;132(3):623-630.
 41. Nassif A, Bensussan A, Boumsell L, et al. Toxic epidermal necrolysis: effector cells are drug-specific cytotoxic T cells. *J Allergy Clin Immunol*. 2004;114(5):1209-1215.
 42. Yaseen FS, Saide K, Kim SH, et al. Promiscuous T-cell responses to drugs and drug-haptens. *J Allergy Clin Immunol*. 2015;136(2):474-476.

43. Zanni MP, von Greylers S, Schnyder B, et al. HLA-restricted, processing- and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. *J Clin Invest*. 1998;102(8):1591-1598.
44. Pichler WJ. Immune pathomechanism and classification of drug hypersensitivity. *Allergy*. 2019;74(8):1457-1471.
45. Pan RY, Chu MT, Wang CW, et al. Identification of drug-specific public TCR driving severe cutaneous adverse reactions. *Nat Commun*. 2019;10(1):3569.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Thomson PJ, Kafu L, Meng X, et al. Drug-specific T-cell responses in patients with liver injury following treatment with the BACE inhibitor atabecestat. *Allergy*. 2021;76:1825-1835. <https://doi.org/10.1111/all.14652>