### Supplementary Text

### Lymphocyte Proliferation Assay and PBMC ELIspot

PBMC from study patients (1x105/well) were incubated with atabecestat and its metabolites; [DIAT], *N*-acetyl-DIAT and atabecestat epoxide (12.5-100µM) in cell culture medium for a period of 5 days. Cell culture medium for T-cell experiments was composed of Roswell Park Memorial Institute (RPMI) supplemented with 10% human AB serum, HEPES (25 mM), penicillin (1000 U/mL), streptomycin (0.1 mg/mL), L-glutamine (2 mM) and transferrin (25 µg/mL). [3H] thymidine was added for an additional 16 hours and T-cell proliferation was measured using scintillation counting (MicroBeta 2, Perkin Elmer). PBMCs collected from 2 healthy volunteers, were subjected to the lymphocyte proliferation assay using the same compounds as comparators. To ascertain appropriate study compound concentrations for testing PBMC toxicity was assessed using trypan blue dye exclusion. Subsequently the concentrations of the compounds that inhibited mitogen-driven PBMC proliferation was measured. Since concentrations of 100 µM did not inhibit proliferation in PBMC all experiments were conducted at study compounds concentrations of 100microM and below.Secretion of IFN-γ and granzyme B from patient and healthy donor PBMC was visualised using the ELIspot assay. ELIspot plates (Merck Millipore, Tullagreen, Cork, IRL) were activated with 15 µL of 35% ethanol and washed 5 times with dH2O. The plates were then coated with 100 µL/well of IFN-γ and Granzyme B capture antibodies (15 µg/mL) and incubated overnight at 4°C. The following day, wells were washed and then blocked with medium at room temperature for a period of 30 minutes. Next, PBMCs (5x105/100 µL) were incubated in the presence of atabecestat and its metabolites (DIAT, *N*-acetyl-DIAT and atabecestat epoxide) at concentrations of 0-50 µM. Following incubation, the ELIspot plate was developed for IFN-γ and granzyme B according to the manufacturer’s instructions and spots were counted using an AID ELIspot reader (Cadama Medical, Stourbridge, UK).

### Generation of study compound-specific T-cell clones

T-cell cloning was conducted to atabecestat and DIAT in patients 1, 4, 5, 6, 7, 9, 12 and 13 and to atabecestat epoxide and *N-*acetyl DIAT in patients 4, 6 and 12. Drug-responsive T-cell lines were generated via the culture of subject PBMC in the presence of the study compounds for a period of 14 days. IL‑2 (100 U/mL) was added on days 6 and 9 to maintain cellular proliferation. On day 14, T‑cells were cloned via means of serial dilution over a course of 4-8 weeks. Briefly,  cells from each individual T-cell line were seeded at 0.3–3 cells/well into 96-well round-bottomed plates and restimulated with 2.5×104 allogeneic irradiated PBMC and PHA (1 μg/ml). Clones were maintained in IL-2 containing medium and two weeks later, well-growing clones were harvested, propagated, and tested for atabecestat (metabolite) responses. The specificity of T-cell clones was assessed by culturing T-cell clones (5x104/50 µL) with autologous epstein-Barr virus (EBV)-transformed B-cells (1x104/50 µL) in the presence and absence of the study compounds for a period of 48 hours (37°C; 5% CO2). EBV-transformed B-cell lines were produced with supernatant from virus producing B95.8 cells. Following incubation, [3H] thymidine (0.5 µCi) was added for a further 16 hours and cellular proliferation was measured via scintillation counting. Clones which yielded a stimulation index of 1.5 or greater were subjected to mitogen expansion as described above in the presence of IL-2 for a period of 14 days prior to assays to determine phenotype and functionality. All phenotyping analyses were conducted in the first 4 weeks after clone generation.

**Phenotype and function of study compound-responsive T-cell clones**

Flow cytometry was used to characterize the phenotype of the T-cell clones. T-cell clones (5x104/50 µL) were initially stained with CD4-Fluorescein isothiocyanate/ Allophycocyanin (FITC/APC) (3 µL) and CD8-Phycoerythrin (PE) (3 µL) and incubated at 4°C for 20 minutes prior to being analyzed. The expression of T-cell receptor (TCR)-Vβ was analyzed in T-cell clones by staining using an 8 tube panel, containing a total of 24 monoclonal antibodies, known to bind specific TCR-Vβ subtypes (IO Test Beta Mark TCR Vβ Repertoire Kit) (Beckman Coulter, Brea, CA, USA). Chemokine cell surface markers were analyzed using the following antibodies; CCR2-PE, CCR1-PE, CCR4-PE, CCR8-PE CXCR6-PE, E-cad-PE, CXCR3-APC, CXCR1-APC, CCR9‑APC, CCR6-APC, CCR3-FITC, CLA-FITC, CD69-FITC (BD Biosciences (Oxford, UK). Approximately 5x104 T-cell clones were stained for the cell surface markers using the directly conjugated antibodies listed above (maximum of three/tube), with cells on ice for 20 minutes. Expression of all cell surface receptors was analyzed using a FACSCanto II flow cytometer. A total of 10,000 events were acquired for each sample.

Dose-dependent proliferative responses and cross-reactivity with other study compounds was assessed by culturing T-cell clones (5x104/50 µL) with autologous EBV-transformed B-cells (1x104/50 µL) in a U-bottomed 96 well plate in the presence and absence of atabecestat, atabecestat epoxide, DIAT and *N-*acetyl DIAT (0-100 µM). The plates were incubated (37°C, 5% CO2) for 48 hours, with [3H] thymidine added for an additional 16 hours. Cellular proliferation was measured via scintillation counting. In the case of some T-cell clones secretion of IFN-γ was used as a readout via the ELIspot assay.

The cytokine secretion by T-cell clones was assessed using ELIspot plates coated with antibodies specific for the following cytokines; IFN-γ, granzyme B, IL-13, IL-5, IL-17, IL-22, IL-10 and perforin. Following overnight incubation, the ELIspot plate was blocked and T-cell clones were co-incubated with autologous EBV-transformed B-cells in the presence and absence of the study compounds (37°C, 5% CO2). Following 48-hour incubation, the ELIspot plate was developed as described above.

Immune-mediated T-cell killing of autologous antigen presenting cells was assessed through the use of a [51Cr] release assay. Briefly, [51Cr] loaded antigen presenting cells were incubated with clones in the presence or absence of study compounds (atabecestat or DIAT) for 4 h. Specific lysis was calculated as 100 × (experimental release − spontaneous release)/(maximal release − spontaneous release).

**TCR sequencing**

Total RNA was extracted from the T-cell clone lysates with the Qiagen RNeasy mini kit according to manufacturer’s recommendation and 1 ng (or up to 3 µl elution volume) of total RNA was used as input material for a 384-well plate based single cell TCR sequencing platform which is based on the workflow as described in Han et al., 2014 (1). Briefly, TCRα and TCRβ transcripts are reverse transcribed and cDNA is amplified in 3 consecutive PCR reactions using various TCRα and TCRβ specific primers located in the variable and constant region of the receptor sequence. Individual wells are barcoded during the last PCR step, PCR products are pooled and purified using gel purification on a Pippin instrument and subsequenct Ampure bead purification steps. Next, TCRα and TCRβ amplicons are sequenced on an Illumina MiSeq instrument with 2 x 300bp V3 chemistry. Output fastQ files are used as input for a custom TCR preprocessing pipeline that after several QC steps aligns the QC passing reads to the IMGT database to annotate the V(D)J-regions and finally outputs the detected TCR information. For this analysis, all TCRα and TCRβ sequences with fraction > 0.05 of total reads were retrieved (minimal total sequence reads > 500).

## Pathways of activation of T-cell clones and the involvement of antigen presenting cells in the study compound-specific T-cell response

In initial experiments the autologous EBV-transformed B-cells were omitted from the T-cell proliferation assays. Subsequently, EBV-transformed B-cells were pulsed for periods of 1 or 16 hours with atabecestat (12.5 µM) or DIAT (100 µM), prior to washing 3 times to remove the unbound drug. The EBV-transformed B-cells were then irradiated and co-incubated (1x104/50 µL) with T-cell clones (5x104/50 µL) in the absence of soluble drug for a period of 48 hours, (37°C, 5% CO2). T-cell activation was quantified via proliferation measured using [3H] thymidine incorporation. T-cell clones incubated with unpulsed autologous EBV-transformed B‑cells in the presence and absence of the free drug were used as controls. EBV-transformed B-cells were also fixed with glutaraldehyde, prior to quenching with glycine and 3 washing steps to prevent antigen processing. T-cell clones were incubated with fixed EBV-transformed B-cells in the presence and absence of the study compounds for a period of 48 hours (37°C, 5% CO2). T-cell activation was quantified via proliferation measured using [3H] thymidine incorporation. MHC restriction of T-cell responses was assessed by culturing the T-cell clones, EBV-transformed B-cells and the study compounds in the presence of anti-human MHC class I (Human Leukocyte antigen [HLA]‑A, B and C), MHC class II (HLA-DP, DQ and DR) or isotype control class I and II antibodies (BD Pharmingen, San Jose). T-cell proliferative responses were measured using [3H] thymidine incorporation and IFN-γ ELIspot. Where available, individual MHC class II allele blocking (HLA-DP, DQ and DR) was conducted using the method described above.

**Reference**

1. Han A, Glanville J, Hansmann L, Davis MM. Linking T-cell receptor sequence to functional phenotype at the single-cell level. Nat Biotechnol. 2014;32(7):684-92.

**Supplementary Table 1. HLA typing of hypersensitive patients**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | HLA-A1 | HLA-B | HLA-C | HLA-DQB1 | HLA-DRB1 |
| Patient 5 | 01:01:01 | 02:01:01 | 07:02:01 | 51:01:01 | 01:02:01 | 07:02:01 | 03:02:01 | 06:02:01 | 04:01:01 | 15:01:01 |
| Patient 6 | 02:01:01 | 03:01:01 | 14:02:01 | 44:02:01 | 05:01:01 | 08:02:01 | 03:01:01 | 06:09:01 | 12:01:01 | 13:02:01 |
| Patient 7 | 02:01:01 | 68:01:01 | 07:02:01 | 35:01:01 | 04:01:01 | 07:02:01 | 03:02:01 | 06:02:01 | 04:04:01 | 15:01:01 |
| Patient 9 | 01:01:01 | 32:01:01 | 08:01:01 | 14:01:01 | 07:01:01 | 08:02:01 | 03:02:01 | 06:02:01 | 04:04:01 | 15:01:01 |

**Supplementary Table 2. Summary of the number of study compound-responsive T-cell clones generated from PBMC of the atabecestat DILI patients.**

|  |  |  |
| --- | --- | --- |
|  | ***T-cell cloning to atabecestat*** | ***T-cell cloning to DIAT*** |
| **Patient ID** | **Tested number of clones** | **Number of drug-responsive clones (test one)** | **Number of drug-specific clones (repeat testing)** | **Percentage of responding clones (%)** | **Tested number of clones** | **Number of drug-responsive clones (test one)** | **Number of drug-specific clones (repeat testing)** | **Percentage of responding clones (%)** |
| Patient 1 | 183 | 0 | 0 | 0 | 500 | 16 | 3 | 0.6 |
| Patient 4 | 37 | 1 | 0 | 0 | 36 | 3 | 0 | 0 |
| Patient 5 | 48 | 2 | 2 | 4.17 | 58 | 4 | 1 | 1.72 |
| Patient 6 | 99 | 5 | 2 | 2.02 | 189 | 19 | 4 | 2.12 |
| Patient 7 | 38 | 0 | 0 | 0 | 19 | 0 | 0 | 0 |
| Patient 9 | 57 | 0 | 0 | 0 | 56 | 2 | 1 | 1.79 |
| Patient 12 | 46 | 0 | 0 | 0 | 22 | 1 | 1 | 4.54 |
| Patient 13 | 16 | 0 | 0 | 0 | 11 | 0 | 0 | 0 |
|  |  |  |  |  |  |  |  |  |
|  | ***T-cell cloning to acetyl DIAT*** | ***T-cell cloning to atabecestat epoxide*** |
| **Patient ID** | **Tested number of clones** |  | **Number of drug-specific clones** | **Percentage of responding clones (%)** | **Tested number of clones** |  | **Number of drug-specific clones** | **Percentage of responding clones (%)** |
| Patient 4 | 29 | 0 | 0 | 0 | 20 | 1 | 0 | 0 |
| Patient 6 | 132 | 9 | 3 | 2.27 | 111 | 3 | 0 | 0 |
| Patient 12 | 20 | 0 | 0 | 0 | 23 | 5 | 0 | 0 |

**Supplementary Table 3. HLA typing of hypersensitive patients**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Patient ID** | **Patient 6** | **Patient 6** | **Patient 5** | **Patient 6** | **Patient 5** | **Patient 5** |
| **Sample ID** | **DIAT TCC 1** | **DIAT TCC 2** | **DIAT TCC 3** | **Atabecestat TCC 1** | **Atabecestat TCC 2** | **Atabecestat TCC 3** |
| *TCRα clonotype* |  |  |  |  |  |  |
| TRACDR3aa\_1 | CADVRA\_SEKLVF | CAVRPEYNQGGKLIF | CAMRDDTGGFKTIF | CAMREGRSGGTSYGKLTF | CAMRGATGGFKTIF | CIVRVVSSGSARQLTF |
| TRACDR3aa\_2 | CAAGSSNTGKLIF | NA | CAGRGQGGSEKLVF | CAVEDQGGGNKLTF | CVVSGPASGGSYIPTF | NA |
| *TCRβ clonotype* |  |  |  |  |  |  |
| TRBCDR3aa\_1 | CASSPQGVPEQFF | CASSRETGMNTEAFF | CASSLDLGTYADTQYF | CAWTTGTGNGYTF | CSASGDSYEQYF | CASSLTGQGHMRGYTF |
| TRBCDR3aa\_2 | NA | NA | NA | CASSLGGGAIGYTF | NA | CASSEKDTDTQYF |