# **Culture media.**

Cell culture medium for T-cells (R9) is composed of RPMI supplemented with 10% human AB serum, HEPES (25mM), penicillin (1000 U/mL), streptomycin (0.1mg/mL), L-glutamine (2mM) and transferrin (25µg/mL). Epstein-barr virus (EBV) -transformed B-lymphoblastoid cell lines were cultured in F1 medium composed of RPMI supplemented with 10% foetal bovine serum, HEPES (25mM), penicillin (1000 U/mL), streptomycin (0.1mg/mL) and L-glutamine (2mM).

**Synthesis of 6-amino substituted abacavir analogues.**

The initial stage involved the synthesis of a stock of intermediate 1. From this chiral intermediate the synthesis of seventeen target molecules (A-Q) was conducted (Supplementary Figure 1). For analogues A-N, intermediate 1 (150.00 mg, 564.55 µmol, 1.00 *eq*), azetidine variants (2.00 *eq*) and N, N-diisopropylethylamine (145.92 mg, 1.13 mmol, 2.00 *eq*) were taken up into a microwave tube in isopropyl alcohol (2.00 mL). The sealed tube was heated at 70 °C for 2 hours under microwave. LC-MS showed that the starting material was consumed completely. The mixture was concentrated in vacuum to give crude product. The crude product was then purified by thin-layer chromatography. Analogues O-Q were synthesized following the same procedure with the azetidine variants replaced with derivatives of the amino group.

**Donor characteristics and T-cell cloning.**

Four HLA-B\*57:01+ donors were selected from the Liverpool Centre for Drug Safety Science cell bank containing peripheral blood mononuclear cells (PBMCs) from 1200 genotyped healthy donors. Approval for the study was obtained from the Liverpool research ethics committee and informed consent was received from participants prior to inclusion in the study. PBMCs were incubated in the presence of abacavir, or analogues G, H, 15 (from our previous study (1)) or J (35µM), in R9 medium for a period of 14 days. On days 6 and 9 cells were fed with R9 medium containing recombinant human IL-2 (100U/mL) to preserve the drug driven expansion of T-cells. On day 14, CD8+ T-cells were positively selected using MultiSort kits (Miltenyi Biotec, Surrey UK) and T-cell clones were generated via means of serial dilution and phytohaemagglutinin (PHA; 5µg/mL) stimulation. T-cells were fed every 2 days with R9 medium containing IL-2 (100U/mL) and growing clones were transferred to a new 96 well plate and expanded across 4 wells. Clones were restimulated and further expanded every 14 days.

**Drug-specific T-cell responses.**

Specificity of CD8+ clones was measured by culturing T-cells with irradiated autologous EBV-transformed B-cells (as antigen presenting cells; 1x104/well) ± abacavir (35µM). Following a 48 h incubation, [3H] thymidine (0.5µCi) was added and cellular proliferation assessed 16 h later via scintillation counting. CD phenotyping of clones was performed using BD FACSCanto II flow cytometer.

IFN-γ ELIspot was used as a second readout of the drug-specific response to assess dose-dependent T-cell activation and cross-reactivity. Drug-specific clones (5x104, 50µL) were added to ELIspot plates with EBV transformed B-cells (1x104, 50µL) and abacavir (analogues) (10, 20, 50µM; 100µL) for a period of 48 h. Spot forming units (cytokine-secreting T-cells) were visualised and quantified using an AID ELIspot reader (Cadama Medical, Stourbridge, UK).

**Modelling of abacavir (analogues) HLA-B\*57:01 binding.**

HLA, HLA binding peptide (HSITYLLPV) and drug ligands (abacavir and analogues D, G, H, M, O, P and Q) were prepared for docking in Spartan’08 (wavefunction inc. Irvine, California, USA: 1991-2009). Native SMA ligand was exported from PDB 3UPR. For each analogue, the cyclopropyl group of abacavir was replaced. Merk molecular force field minimization calculations were performed with all atoms frozen except newly added substitutions. For docking studies GOLD 5.1 (CCDC Software Limited, Cambridge, UK) was used to examine the predicted binding poses of the abacavir analogues within the F-pocket of HLA-B\*57:01, PDB code 3UPR (2). Figures of abacavir analogues predicted binding conformations within HLA-B\*57:01 were produced using PYMOL software version 2.5.

**MHC purification and peptide elution.**

C1R.B\*57:01 are transfectants of C1R cells expressing HLA-B\*57:01 under geneticin selection (3). C1R.B\*57:01 were grown in RF10 [RPMI 1640 (Life Technologies, USA) supplemented with 10% foetal bovine serum (FBS; Sigma, St Louis, USA), 7.5 mM HEPES (MP Biomedicals, Germany), 100 U/mL Pen-Strep (benzyl-penicillin/streptomycin, Life Technologies, USA), 2 mM L-glutamine (MP Biomedicals, Germany), 76μM β-mercaptoethanolamine (Sigma-Aldrich, USA) and 150μM non-essential amino acids (LifeTechnologies, USA)]. HLA expression was maintained in long term culture with 0.5mg/mL geneticin (G418; LifeTechnologies, USA).

C1R.B\*57:01 cells were grown to high density in the presence or absence abacavir and analogues D (activates abacavir-responsive T-cells), H (activates abacavir-responsive T-cells at high concentrations), M (does not activate abacavir-responsive T-cells) and 15 (from our previous study (1) does not activate abacavir-responsive T-cells) (35μM) for a minimum of 4 days, prior to washing in PBS, pelleting and snap freezing in liquid nitrogen. Cell pellets of 4-5x108 cells were lysed by mechanical and detergent based lysis, the lysates cleared by ultracentrifugation, and HLA class I complexes isolated by immunoaffinity purification using solid-phase bound pan class I antibody W6/32 as described previously (4). Complexes were dissociated using 10 % acetic acid and fractionated by Reversed Phase High Performance Liquid Chromatography (HPLC) on a 4.6mm internal diameter x 100mm monolithic reversed-phase C18 HPLC column (Chromolith SpeedROD; Merck Millipore) using an ÄKTAmicro HPLC (GE Healthcare) system. The peptide/MHC mixture was loaded at 1mL/min onto the column in 98% Buffer A (0.1% Trifluoroacetic acid) and 2% Buffer B (80% Acetonitrile, 0.1% trifluoroacetic acid), and bound material eluted by running a gradient of buffer B at 2ml/min of 2-15% over 0.25 minutes, 15-30% over 4 minutes, 30-40% over 8 minutes, 40-45% buffer B over 10 min, with collection of 500µL fractions. Peptide containing fractions were vacuum concentrated, pooled into 9-12 pools, and reconstituted in 0.1% formic acid.

**Mass spectrometric analysis.**

Reconstituted fraction pools were analysed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) via a data dependent acquisition strategy using a NanoUltra cHiPLC system (Eksigent) coupled to an SCIEX 5600+ TripleTOF mass spectrometer equipped with a Nanospray III ion source. Samples were loaded onto a pre-equilibrated cHiPLC trap column (3µm, ChromXP C18CL, 120 Å, 0.5 mm x 200 µm), at 5µL/min in 0.1% formic acid, 2% acetonitrile, and separated over a cHiPLC column (3µm, ChromXP C18CL, 120 Å, 15cm x 75µm) using a linear gradient of 2-35% Buffer B (80% acetonitrile, 0.1% formic acid)/Buffer A (0.1% formic acid) over 75 minutes at a flow rate of 300nL/min. Data acquisition occurred with the following instrument parameters: ion spray voltage, 2,400 V; curtain gas, 30 l/min; ion source gas, 20 l/min; and interface heater temperature, 150 °C. MS/MS switch criteria selected the top 20 ions meeting the following criteria per cycle: *m*/*z* >200 amu, charge state of +2 to +5, intensity >40 counts per second. After two selections for fragmentation, ions were ignored for 30 seconds. For assignment, MS/MS spectra were searched against the human proteome (UniProt/Swiss-Prot accessed November 2017) using ProteinPilot™ software (version 5.0, SCIEX), considering biological modifications and utilising a decoy database for false discovery rate (FDR) calculations. Identifications were filtered for peptides seen in HLA class I immunoaffinity purifications from parental C1R (includes contaminants and peptide binders of endogenous HLA-B\*35:03 and HLA-C\*04:01), class II purifications, common contaminants of MHC pull downs observed in the lab, as well as peptides derived from the HLA proteins themselves (Supplementary table 1).

**HLA-B\*57:01 immunopeptidome analysis.**

To define the global peptide binding motif under different conditions, distinct peptides assigned by ProteinPilot with a confidence above a local 5% FDR cut-off and delta mass < 0.05 were considered (Supplementary table 2). Non-redundant sequences were used to calculate the prevalence of peptides of each length, and of nine amino acid peptides possessing specific residues at the primary anchor positions (P2 and PΩ). To compare peptide presentation across the conditions, and filter for peptides of the constitutive HLA-B\*57:01 repertoire, a combined list of peptides identified with a confidence above a 5% FDR cut-off in at least one data set was used to interrogate all data sets (Supplementary table 3). Where assignments were made in multiple data sets at a confidence above the 5% FDR cut-off, the mean and SD of the retention time was calculated. If a peptide assignment was made below the 5% FDR cut-off, it was considered valid if the retention time was within 5 minutes of the mean 5% FDR retention time. Assignments where the SD of the 5% FDR retention time was greater than 2.5 were excluded. Abacavir and analogue treated data sets (non-redundant by sequence) were filtered (by sequence, modifications not considered) of constitutive ligands identified in the retention time validated untreated data sets as well as those described previously (5). Sequence features of the retention time validated and filtered data sets were visualised using Icelogo software (6) (percentage difference to human proteome using static reference method) at P1 to 3 and PΩ-2 to PΩ of 9-12mer peptide ligands.

**Antiviral activity.**

The antiviral activity of the abacavir analogues was measured using cell viability assays carried out by the Wuxi App Tech company in Shanghai. The antiviral activity was measured on the basis that HIV kills MT-4 cells meaning cell survival in the presence of an analogue was a measure of antiviral activity. The antiviral activity of the abacavir analogues was quantified as EC50 and classified into four categories: good antiviral activity (>10µM), retained antiviral activity (10-60µM), little antiviral activity (60-500µM) and no antiviral activity (>500µM).

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