Pilot study of BK virus-specific T cells for immunotherapy of progressive multifocal leukoencephalopathy: Supplemental Materials

Table of Contents

Supplemental Methods

PyVST Manufacturing and Release. Donors underwent 10-15 L unmobilized blood apheresis with Cobe Spectra cell separator device using standard clinical methodology. Leukapheresis products were collected from the donors and processed to enrich for peripheral blood mononuclear cells (PBMC) using standard clinical methodology. Apheresis product was further processed using semi-automatic density gradient separation and cryopreserved or used fresh for initiation of PyVST specific T cell cultures. At culture initiation, the PBMC concentrate was divided into two equal portions, 200x10e6 each, evaluated for viability and suspended in complete culture media (CM) composed of composed of Aim V (Gibco) and 5% AB human serum (CEE, Clinical Center, NIH) supplemented with recombinant (r) IL-7 (500 IU/ml) and rIL-15 (100 IU/ml). One PMBC aliquot was used as antigen presenting or stimulator cells; these were suspended at 50x10e6 total nucleated cells (TNC)/ml and incubated with peptide libraries (pepmixes) of BK large T (LT) and BK VP1 pepmixes at 0·5mcg/ml for 30 minutes and subsequently irradiated (25Gy). Meanwhile, the responder cells were set aside. Finally, responders were co-cultured with stimulators at 1:1 ratio at 1·5x10e6 TNC/ml in G-rex 100 gas-permeable culture chambers and placed in the humidified tissue culture incubator at 37ºC with 5% CO2. After 72 hours cultures were supplemented with CM containing rIL-7, rIL-15 and rIL-2 (30IU/ml). Subsequently, media were exchanged or added every 3 days to maintain viable TNC concentration at 1·5x10e6/ml. On day 14 resulting PyVST cell product was tested for release criteria and harvested, washed and prepared for cryopreservation and/or fresh infusion. Final release criteria testing included appearance by visual check (milky, no aggregates) sterility (no growth at 14 days), mycoplasma PCR negative, endotoxin <5 EU/ml, Gram stain (no organism seen), viability by trypan blue >70%, identity (% CD3+ >60% by flow cytometry).

Final Product Formulation. For fresh infusions, final product contained cells suspended in Plasmalyte-A and 10% HAS at a volume of 30ml, delivered in a syringe. Cryopreserved final product contained cells in freezing media composed of 6% Pentastarch, 5% DMSO, 4% Human Serum Albumin (HAS), 10mcg Dornase alpha and 15 IU/ml heparin per CPS NIH standard operating procedure. On day of infusion product was diluted with Plasmalyte-A and 10 IU/ml heparin.

Magnetic resonance imaging. MRI was performed on 3-tesla scanners per clinical routine at our site and included administration of gadolinium-based contrast material when not contraindicated. 3D T1-Weighted images, T2-weighted fluid-attenuation inversion recovery (FLAIR) images, multislice T2-weighted (T2) and proton density (PD) images, T2*-weighted segmented echo-planar images, and diffusion-weighted images were acquired. When not contraindicated, T1-weighted and T2-FLAIR images were also obtained after intravenous administration of 0.1 mmol/kg gadobutrol. Scans were analyzed qualitatively by a board-certified neuroradiologist (DSR). For quantitative PML lesion analysis, the T1-weighted images were rigidly registered to the Montreal Neurological Institute (MNI)-152 and International Consortium for Brain Mapping nonlinear symmetric 1x1x1mm atlas template (http://nist.mni.mcgill.ca/?p=904). The skull and extracranial tissues were removed, and bias field correction was applied to all available sequences. The FLAIR, T2, and PD images were rigidly co-registered to the T1-weighted image in MNI space, skull-stripped, and bias field corrected in a similar fashion. Subsequently, quantitative longitudinal PML lesion burden measures were generated using consecutive convolutional neural networks applied to the preprocessed T1-weighted, FLAIR, T2 and PD images (when available) as previously described (JCnet)(1).

Flow Cytometry: For cell product phenotyping, a sample from the final product was stained with monoclonal antibodies using standard procedure and analyzed by flow cytometry for the presence of the following markers: viability, CD3, CD4, CD8, CD19 and CD56.

For cell product potency testing, a sample from the day 14 culture was stimulated with BK LT and VP1 pepmixes for 4-5 hours in presence of anti-CD28 and anti-CD49b monoclonal antibodies, brefeldin A and monesin in 48 well plates. Negative control sample was prepared by stimulation with irrelevant peptide library. Positive control was prepared by stimulation with Steptococcal Enterotoxin B (SEB). At the end of the incubation/stimulation period the Intracellular staining was performed using the Cytofix/Cytoperm Fixation/Permebilization kit (BD Biosciences) per manufacturer's recommendations following CPS NIH SOP. Presence of Intracellular IFN-γ and TNFα within $CD4^+$ and $CD8^+$ T cells in viable cells was analyzed by flow cytometry. Data were collected but not used as a part of release criteria in this pilot study.

Samples were acquired using BD LSR/Fortessa Flow Cytometer device. Data were exported and analyzed using FlowJo software.

CD107a/IFNg assay. After thawing, samples of PyVST products were suspended at 10^6 cells/mL in RPMI media (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin sulfate, and 2 mM L-glutamine) and cultured at 200 mL/well in 96-well round plates in a 5% CO_2 incubator at 37°C. To detect BK virus LT- or VP1-specific responses, the cells were stimulated with 1 mg/mL of the appropriate peptide (either BKV LT or VP1 pepmixes) and 1 mg/mL of each of anti-CD28 (BD Biosciences) and anti-CD49d (BD Biosciences). Conjugated CD107a antibody was added to the cells prior to stimulation. After culture for 5 hours in the presence of the secretion inhibitors, GoldiStop (BD Biosciences) and GoldiPlug (BD Biosciences), the cells were washed and surface-stained with antibodies for CD3, CD4, CD8 and CD56 (BD Biosciences). After fixation and permeabilization with Fixation/Permeabilization solution (BD Biosciences) according to the manufacturer's instructions, the cells were intracellularly stained with anti-IFN-g. Flow cytometric analysis was performed using a LSR II (BD Biosciences). The data were analyzed using FlowJo 10.6 software (FlowJo LLC).

Nucleic acid extraction and multiplex qPCR assay. JCV genomic DNA was detected in CSF samples using a CLIA-certified Ultrasensitive Multiplex qPCR assay, which distinguishes the less pathogenic archetype variant typically excreted in the urine from the PML-associated prototype variant found in blood, CSF, and brain in PML (2,3). The assay has a detection limit of 10 copies/ml and targets the highly conserved T antigen coding region for identification and quantitation and the non-coding control region to differentiate archetype from prototype variants.

Supplemental Figures

Figure S1. Development of cGMP-grade PyVST cell product recognizing JCV VPI and LT antigens. Apheresis products from the three healthy donors were used for the validation of the cGMP manufacture of the PyVST cells. Isolated PBMCs were stimulated with BK VP1 and LT pepmixes and cultured in G-Rex containers for 14 days in media containing IL-7, IL-15 and IL-2. A. Protein sequence alignment between BK and JC LT and VP1 antigens obtained using BLAST alignment tool and showing identical (conserved) and conservative aminoacid subsitutions (% positves). B. Fold expansion of viable total nucleated cells (TNC) as compared to day 0 is shown. C. Viability and content of $CD3^+$ T cells in the final product was tested using trypan blue exclusion and flow cytometry, respectively. D. Frequency of PyV-specific T cells in the PyVST cells product has been measured upon recognition of combined VP1 and LT pepmixes, gated on total $CD3^+$ T cells. E. Demonstration of the cross-reactivity of PyVST cells initially generated by priming with BK LT and VP1 pepmixes and the overlapping peptide libraries derived from JC LT and VP1 antigens. PyVST 2 product was in parallel stimulated with indicated BK and equivalent JC pepmixes and analyzed by intracellular detection of TNF-α and IFN-γ in $CD3^+$ T cells.

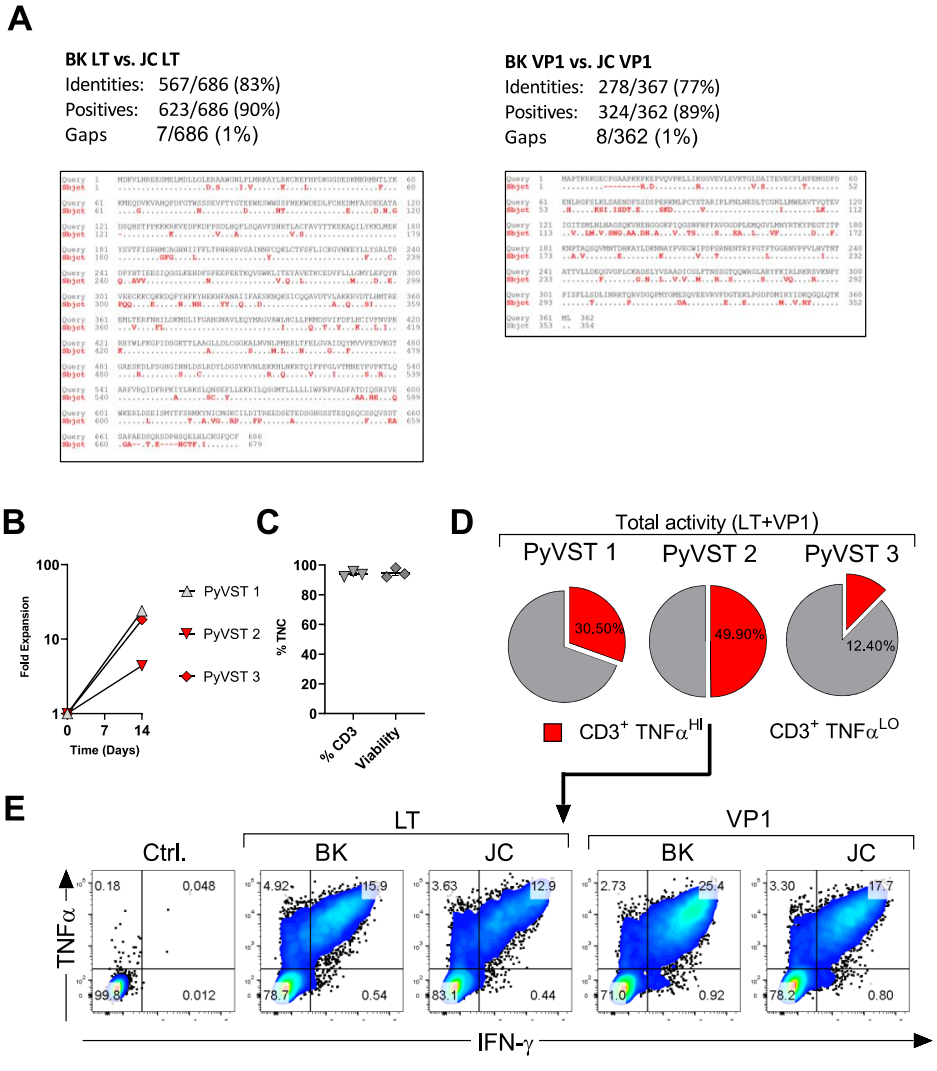


Figure S2. Measurement of cytotoxic activity of PyVST cell products: CD107a/IFNg assay. Degranulation of activated T cells after antigen-specific stimulation results in the polarized mobilization of microtubules that transport lytic granules toward the immunologic synapse formed between CTL and the target. By measuring mobilization of cytolytic granule membrane proteins (lysosome associated membrane proteins; CD107) to the cell surface, which occurs as the granule membrane merges with the cell membrane during degranulation, it is possible to identify and quantiy T-cell degranulation, which is correlated with cytolytic activity, by flow cytometry. We characterized degranulation frequency by measuring CD107a surface expression alone and co-expression of CD107a/intracellular IFNγ following stimulation with BK VP1 and LT pepmixes in a subset of 7 PyVST cell products used for treatment for which clinical samples were available for additional studies. PyVST cell products demonstrate antigen-specific cytolytic activity in both $CD4^+$ and $CD8^+$ T cells.

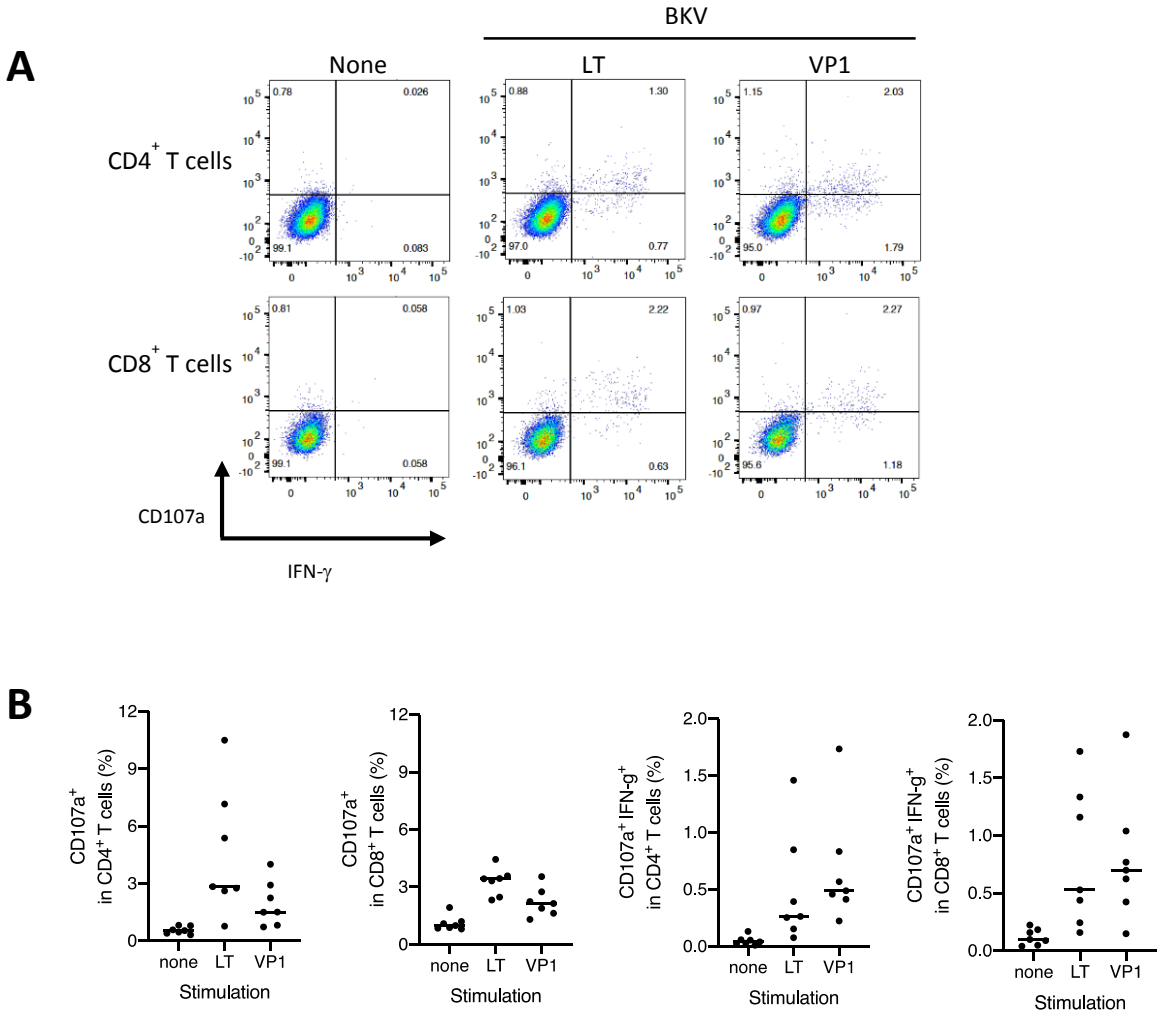


Figure S3. Longitudinal course patient 1
Patient received PyVST from 2 donors: donor 1 ▼ *donor 2* ▼

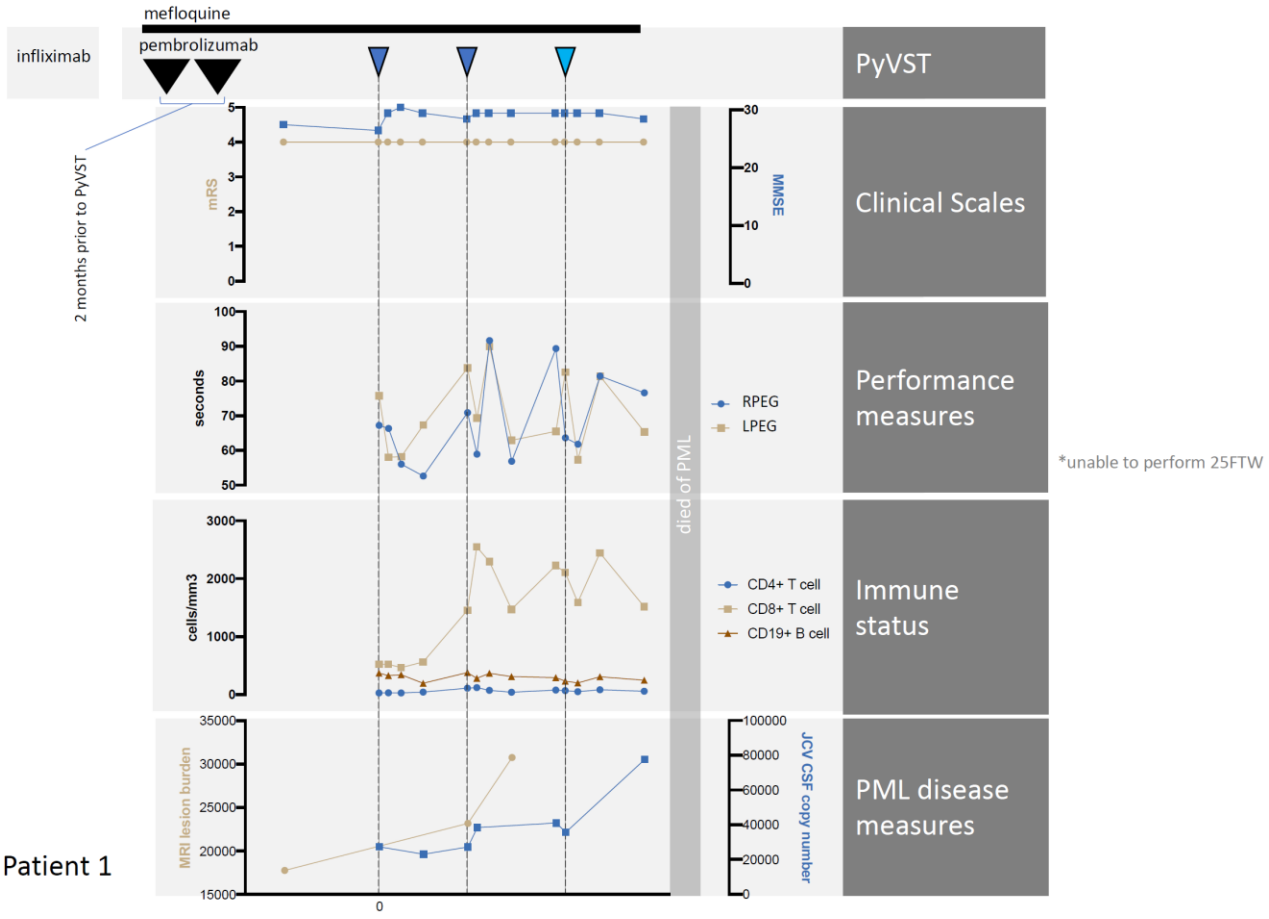


Figure S4. Longitudinal course patient 2

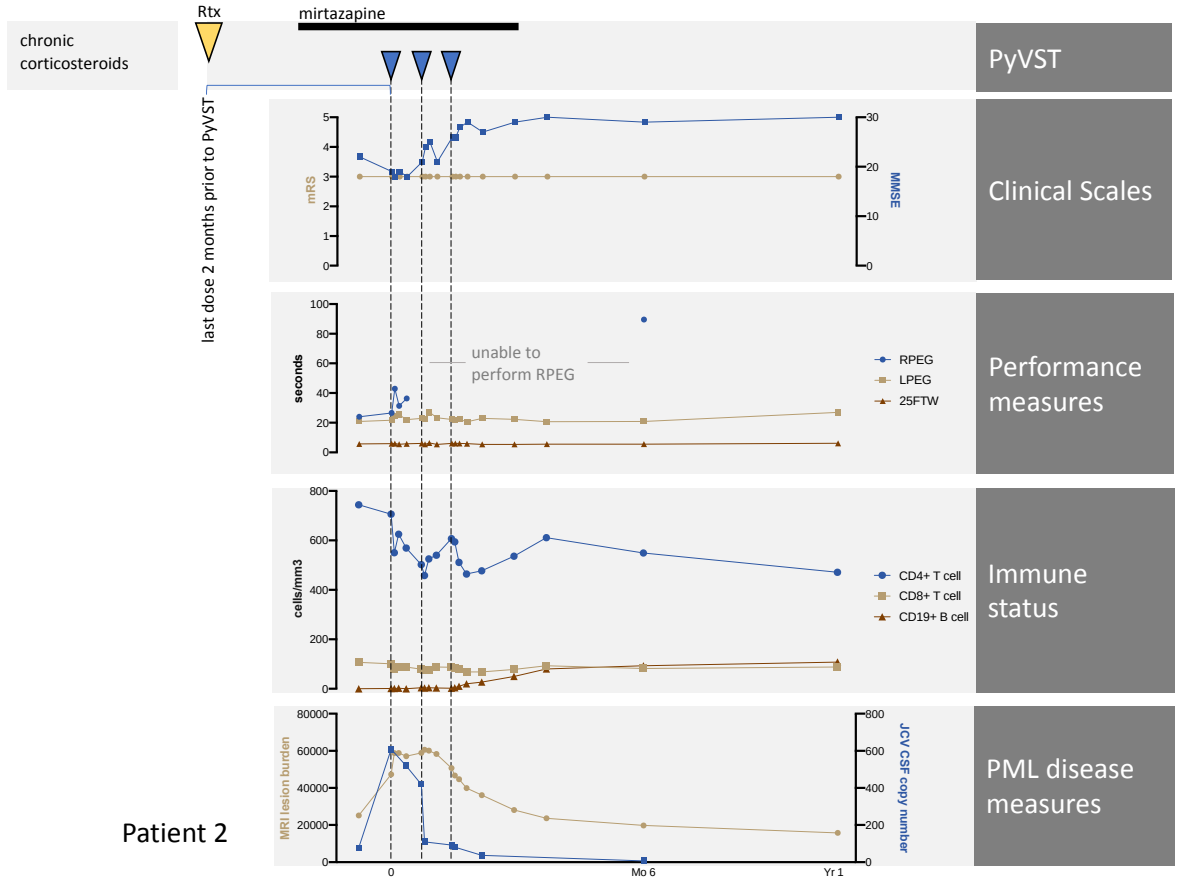


Figure S5. Longitudinal course patient 3

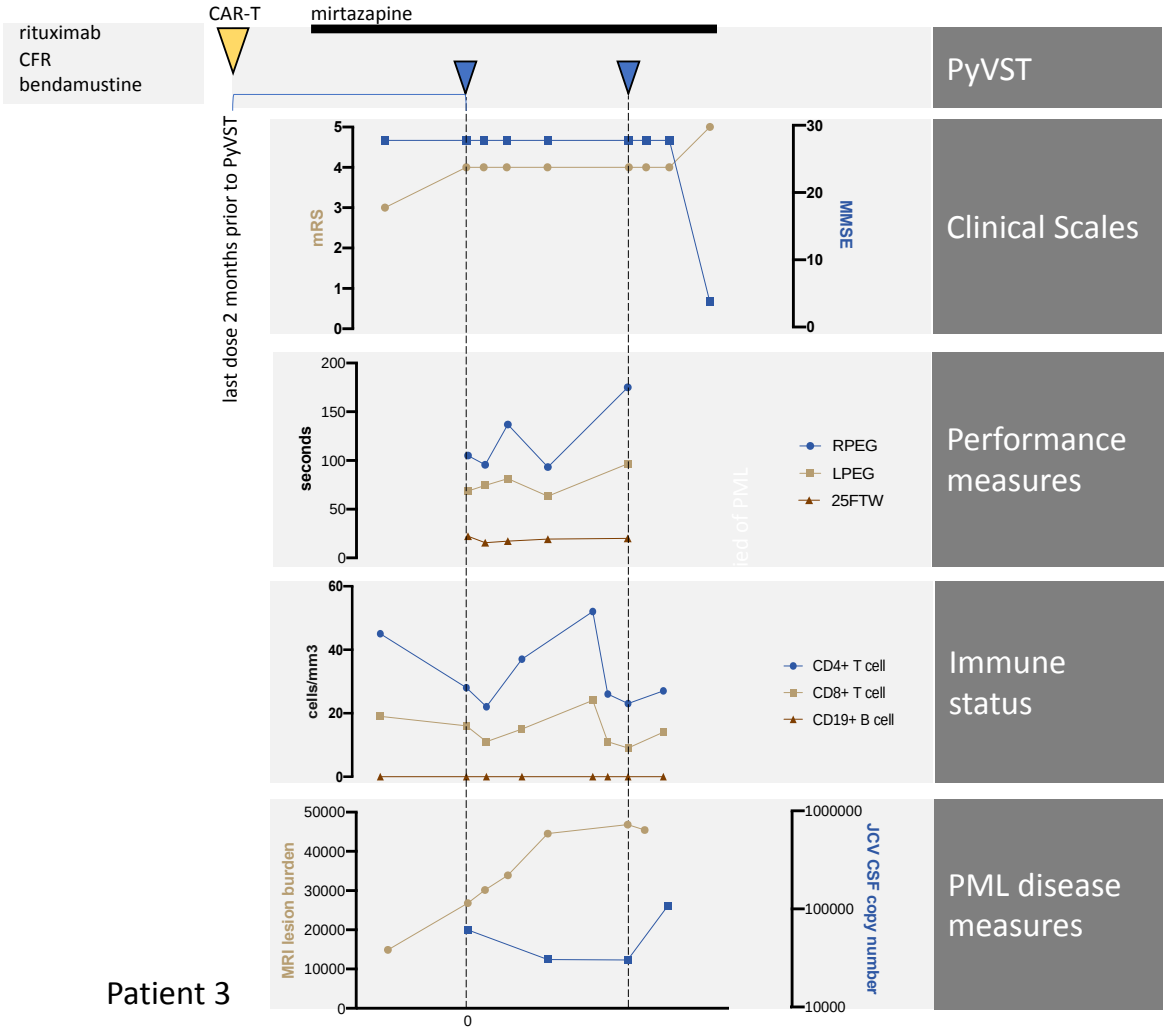


Figure S6. Longitudinal course patient 4

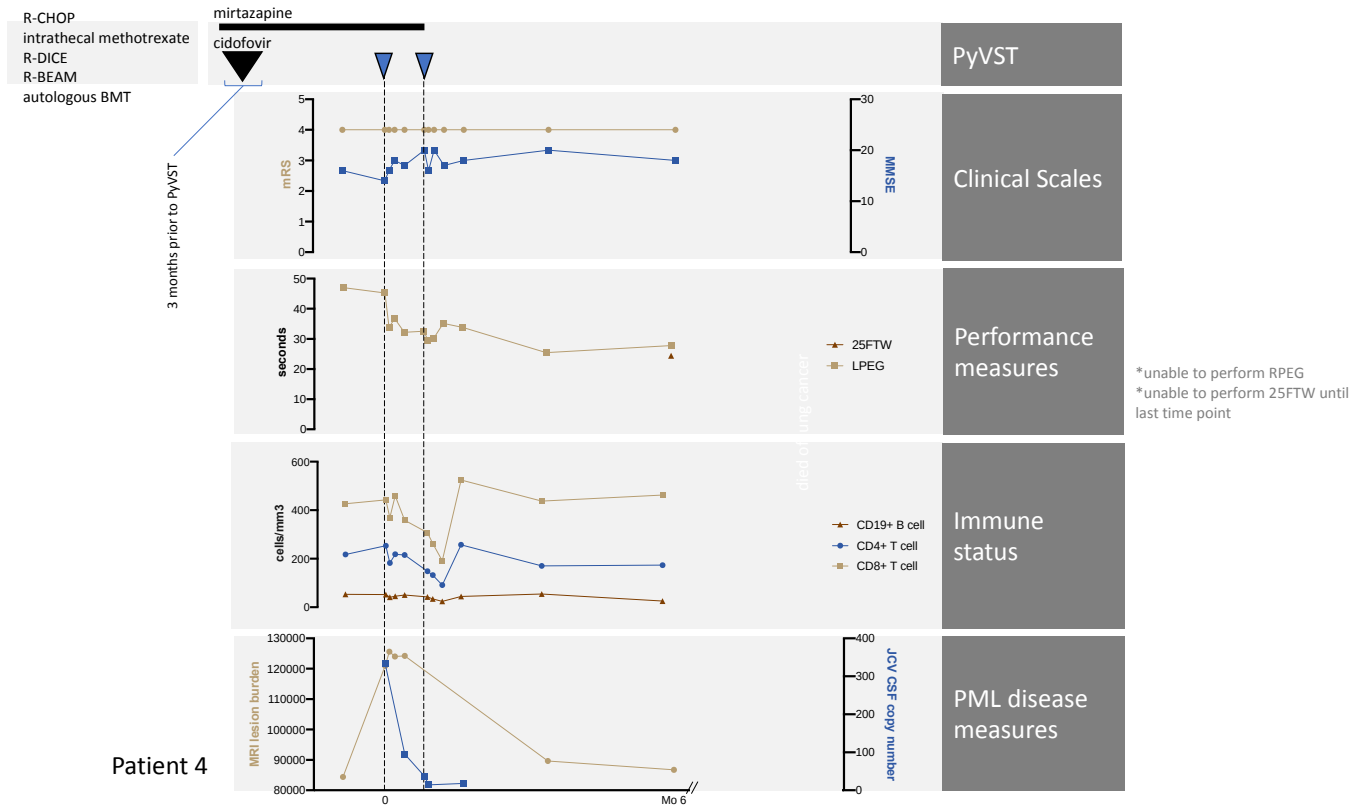


Figure S7. Longitudinal course patient 5
Patient received PyVST from 2 donors: donor 1 ; donor 2

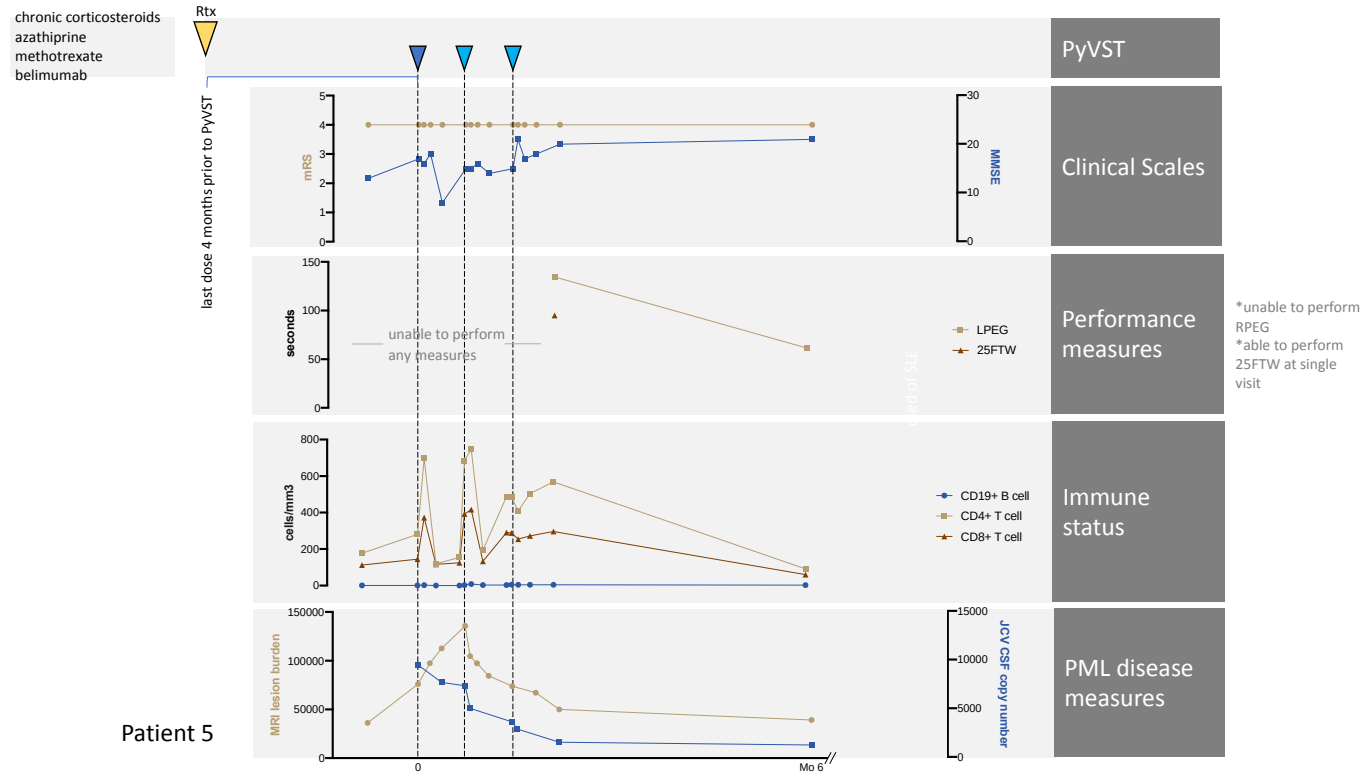


Figure S8. Longitudinal course patient 6

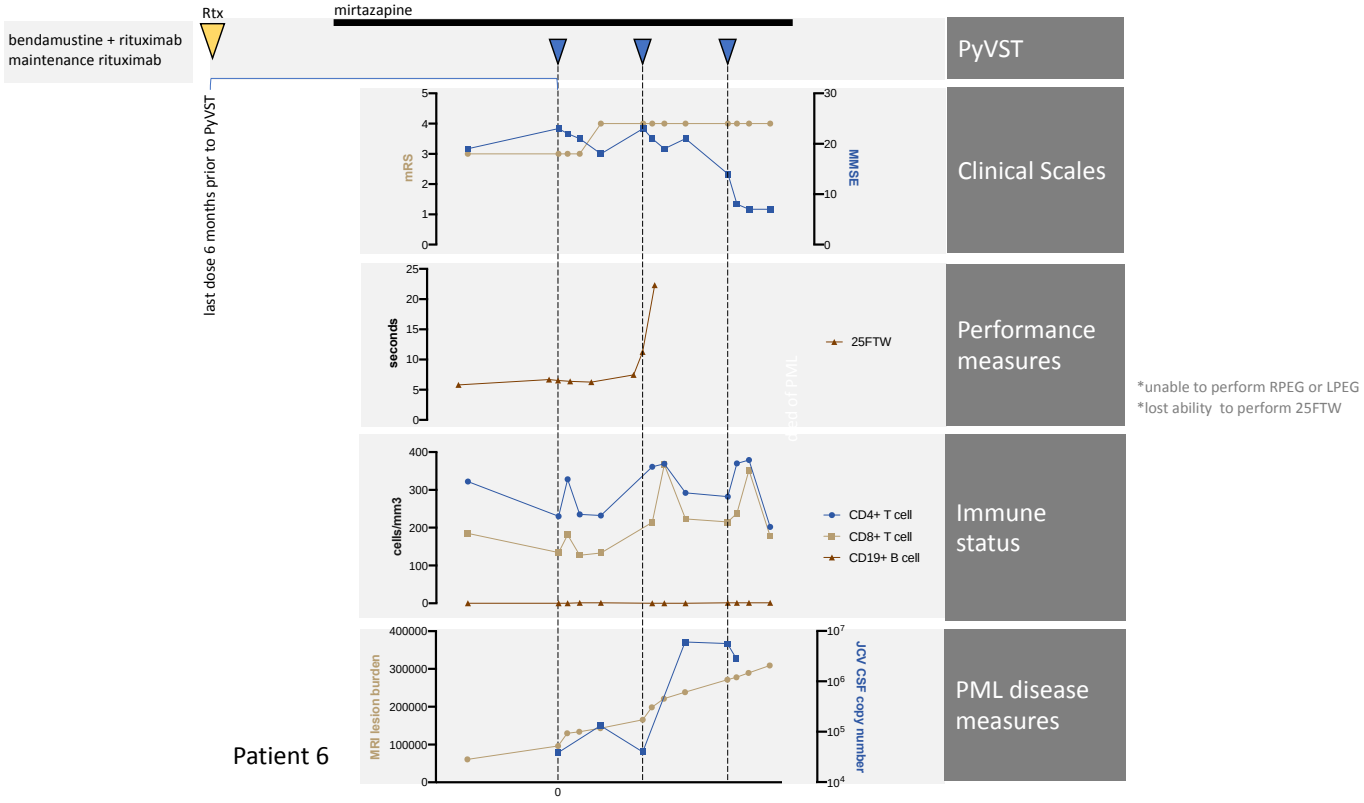


Figure S9. Longitudinal course patient 7

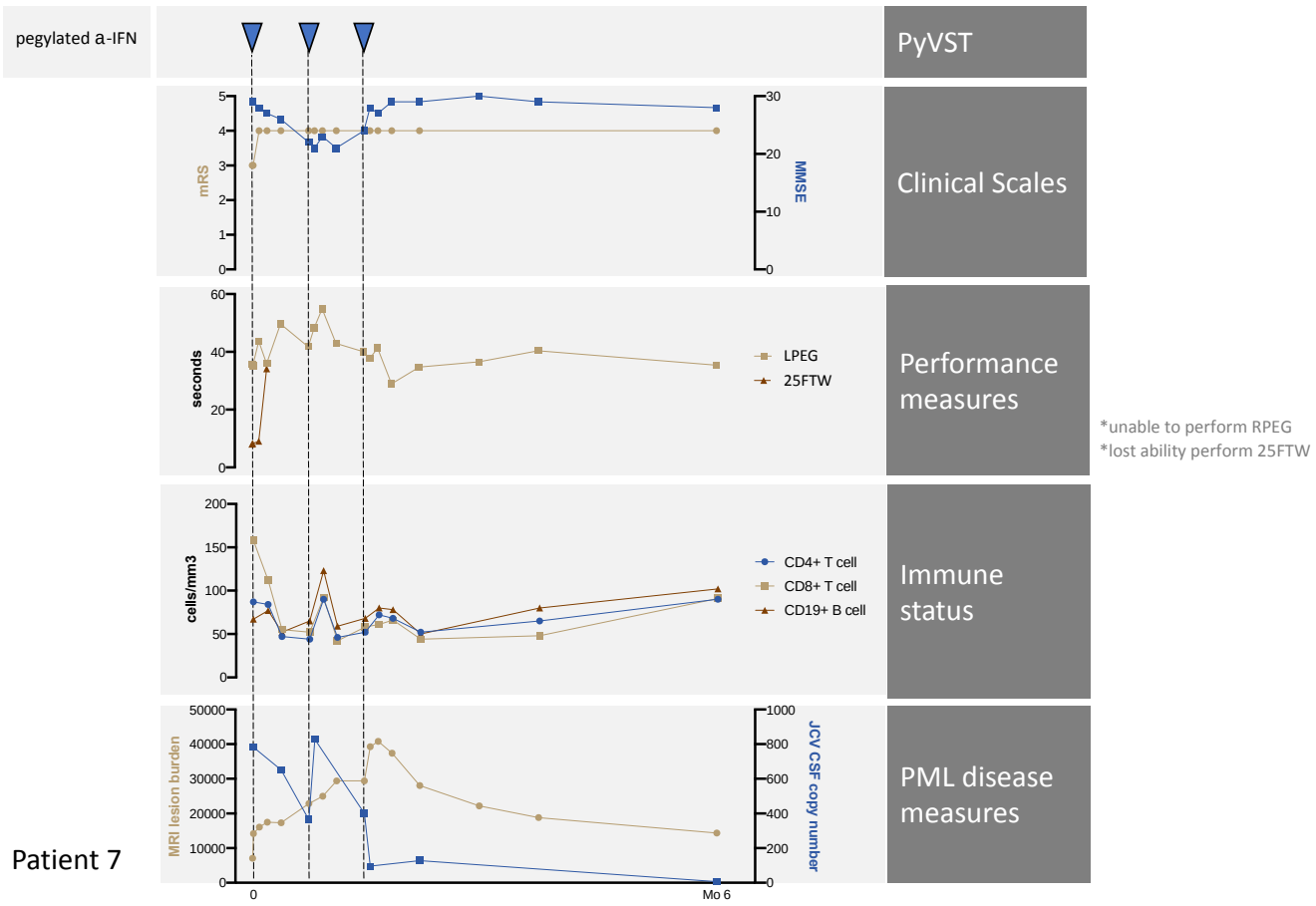


Figure S10. Longitudinal course patient 8

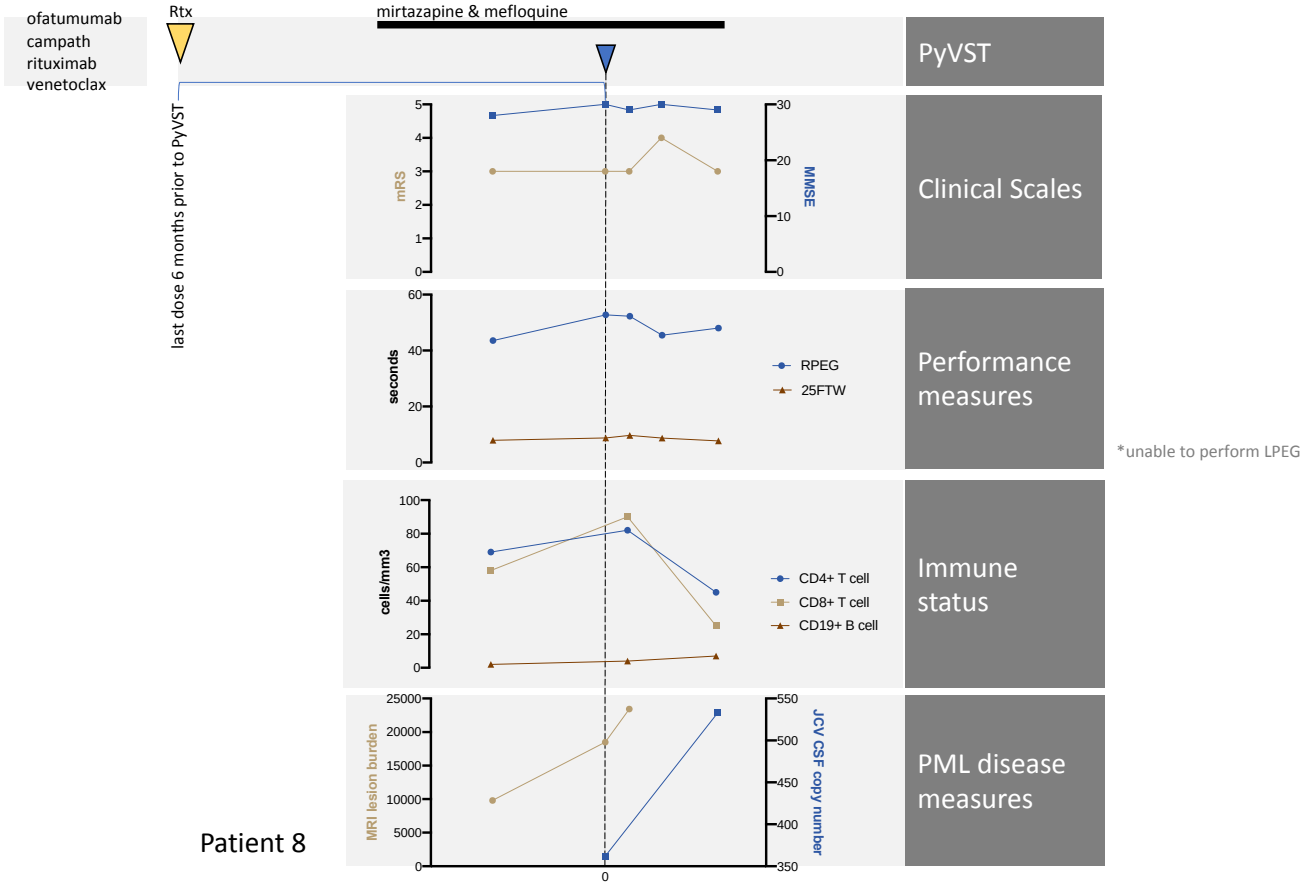


Figure S11. Longitudinal course patient 9

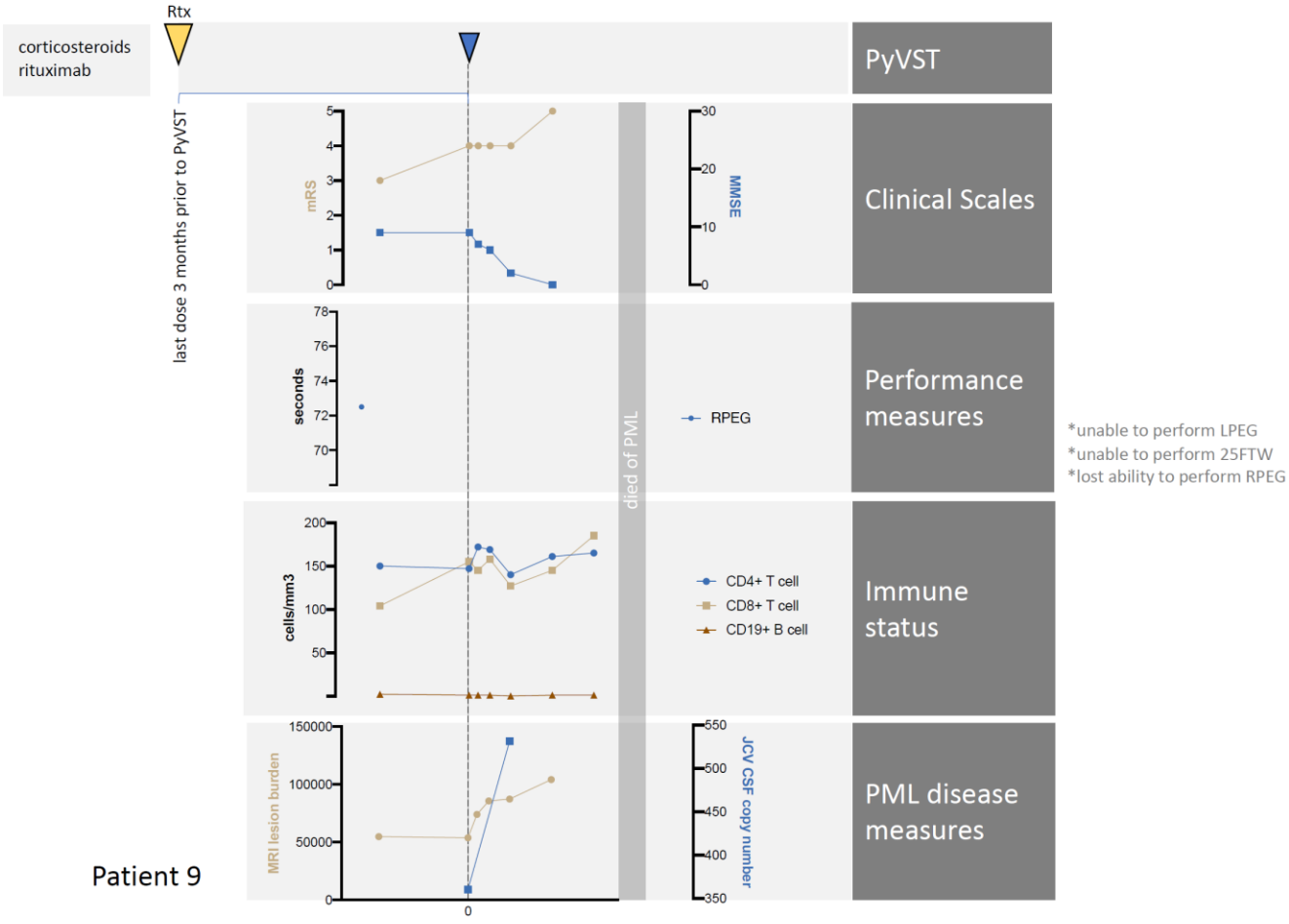


Figure S12. Longitudinal course patient 10

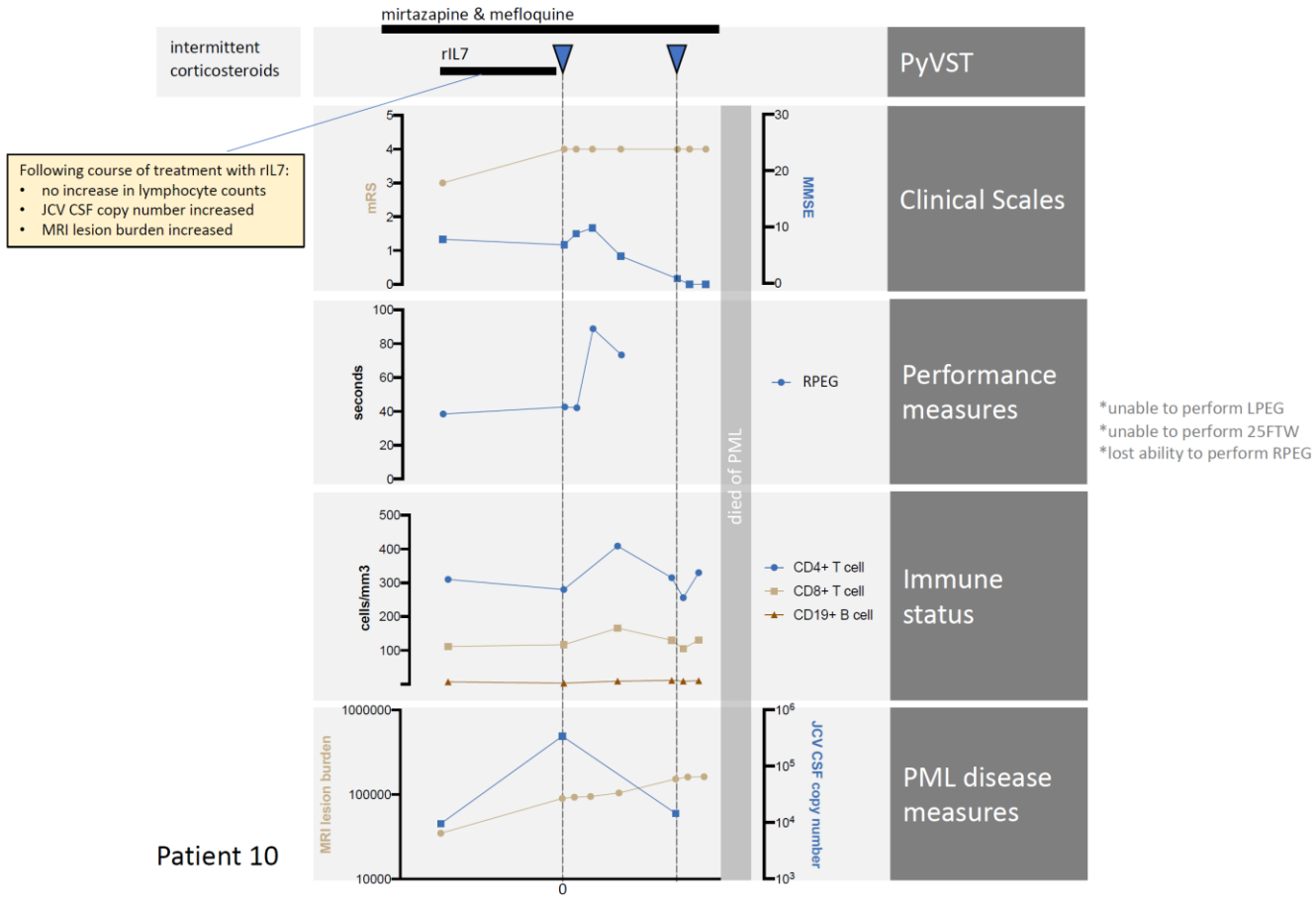
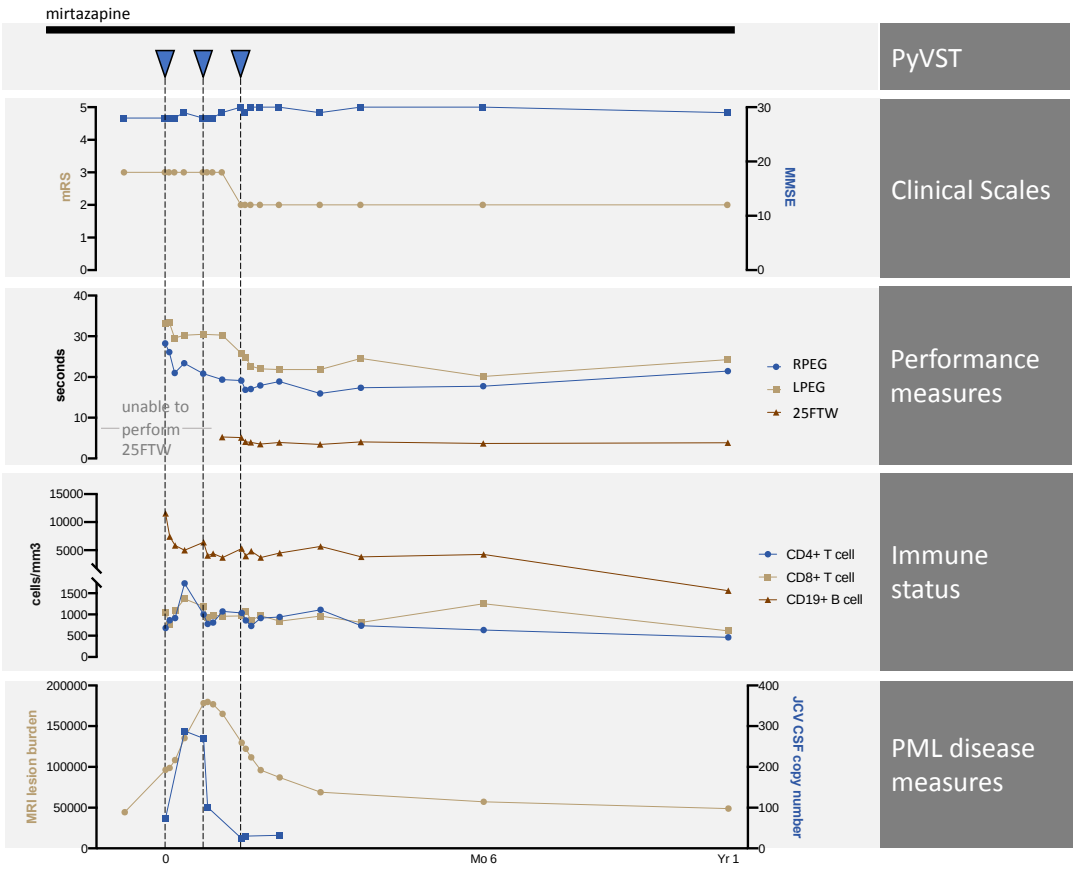
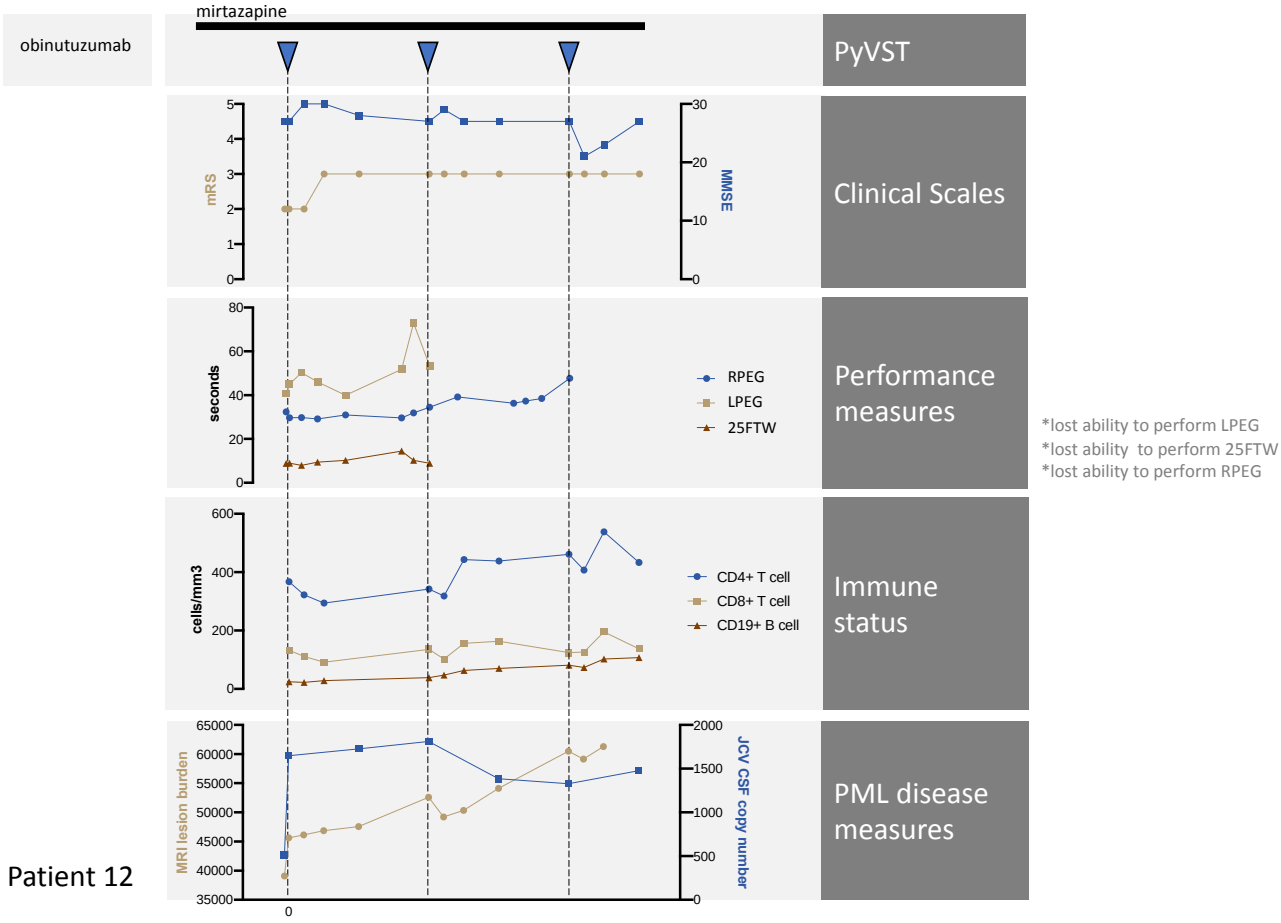


Figure S13. Longitudinal course patient 11



Patient 11

Figure S14. Longitudial course patient 12



Supplemental References

1. Al-Louzi O, Roy S, Osuorah I, Parvathaneni P, Smith B, Ohayon J, Sati P, Pham DL, Jacobson S, Nath A, Reich DS, Cortese I. Progressive multifocal leukoencephalopathy lesion and brain parenchymal segmentation from MRI using serial deep convolutional neural networks. *NeuroImage: Clinical* 2020; **28**, 102499.
2. Ryschkewitsch C, Jensen P, Hou J, Fahle G, Fischer S, Major EO. Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. *J Virol Methods*. 2004; **121**(2):217–21.
3. Ryschkewitsch CF, Jensen PN, Major EO. Multiplex qPCR assay for ultra sensitive detection of JCV DNA with simultaneous identification of genotypes that discriminates non-virulent from virulent variants. *J Clin Virol*. 2013; **57**(3):243–8.