**Supplementary material and methods:**

**Antibodies for Multiparameter Flow Cytometry**

Antibodies used included: TdT (clone HT6, Dako), CD99 (clone TU12, Beckman Coulter, BC), CD2 (clone S5.2, Becton Dickinson, BD), CD5 (clone L17F12, BD), CD7 (M-T701, BD) , CD1a (clone BL6, BD), CD4 (clone RPA-T4, BD), CD8 (clone SK1, BD), CD34 (clone 581, BD), CD38 (clone HB7, BD), TCRαβ (clone IP26A, BC), TCRγδ (clone IMMU510, BC), CD56 (clone NCAM16.2, BD), CD45 (clone HI30, Becton Dickinson), surfaceCD3 (clone SK7, BD), cyCD3 (clone UCHT1, BC), CD335/NKP46 (clone 9E2, BD), CD45RA (clone HI100, BD), CD13 (clone L138, BD), CD33 (clone P67.6, BD), CD117 (clone 104D2, BD), CD19 (BD). Cytoplasmic CD3 and nuclear TdT labeling were performed after IntraStain (Dako, Glostrup, Denmark) permeabilisation. Instrument set-up was regularly calibrated using Calibrite, Rainbows 8 picks, and CST beads systems.

**MDD/MRD assessment by Droplet Digital PCR (ddPCR)**

Quantification of MDD and MRD was performed by ddPCR with the QX100 Droplet Digital PCR system (Bio Rad Laboratories, Hercules, CA). 250ng (5µl) gDNA were amplified in 20µl with 10µl of 2X ddPCR Master Mix (Bio-Rad Laboratories), 1µl of 20X primers and probe (final concentration, 500nmol/l and 200nmol/l). End point PCR was performed on a T100 Thermal Cycler (Bio-Rad Laboratories) followed by droplet analysis on the QX100 reader using QuantaSoft V1.2 software, as per the manufacturer’s recommendations. Each experiment included 2 replicates with no template control (NTC), a positive control using 100ng of diagnostic gDNA, 3 replicates of 250ng of each MDD/MRD sample, 2 replicates of 250ng of a 0.01%/10-4 dilution of diagnostic DNA (corrected for initial infiltration) to evaluate sensitivity and 3 replicates of 250ng pooled PBMC gDNA from 6 healthy donors, to assess non-specific ASO amplification. The final tumour load was calculated as a mean of at least 2 technically acceptable replicates after application of a Poisson correction and exclusion of non-specific channel 2 false positivity. DNA quantity and amplifiability was assessed in a separate reaction by quantification of the albumin housekeeping gene from 100ng of gDNA sample in a single well, total volume 20l.

ddPCR results were analysed using updated criteria defined for Mantle Cell Lymphoma. Briefly, only replicates with ≥9000 droplets were accepted and a single threshold for all samples was set. Positive samples were those with a merge of events ≥3, regardless of the number of positive triplicates. Below quantitative level (BQL) samples had a merge of events of 2. Undetectable samples had, at most, replicates with only one event. If there was a merge of ≥2 events in 3 PBMC with ≥9000 droplets, positivity was corrected as described1. Target quantification was corrected for DNA quantity and quality, based on albumin quantification in a separate reaction1, since the majority of MDD samples were frozen pellets leftover from MFC analysis.

The sensitivity of the selected ASO was at least 0.01% in all cases. Since the quantity of DNA available from the cell pellets was often limited, we compared quantification from triplicates of 250ng (112500 cell equivalents) to 500ng triplicates (225000 cell equivalents) on 10 positive samples, but there was no obvious increased sensitivity with the latter (data not shown) so 250ng triplicates were retained.

Reference:

1 Drandi D, Alcantara M, Benmaad I, Söhlbrandt A, Lhermitte L, Zaccaria G *et al.* Droplet Digital PCR Quantification of Mantle Cell Lymphoma Follow-up Samples From Four Prospective Trials of the European MCL Network. *HemaSphere* 2020; **4**: e347.