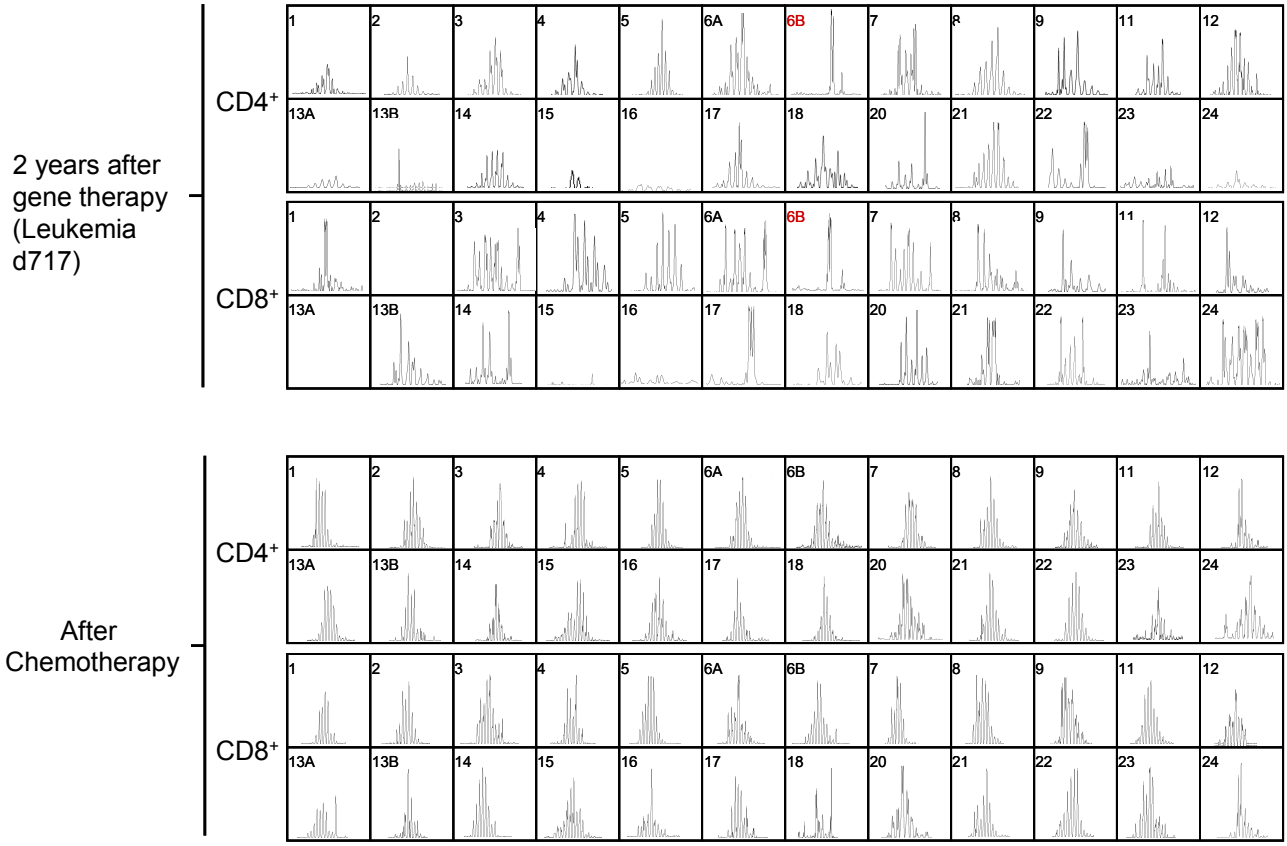
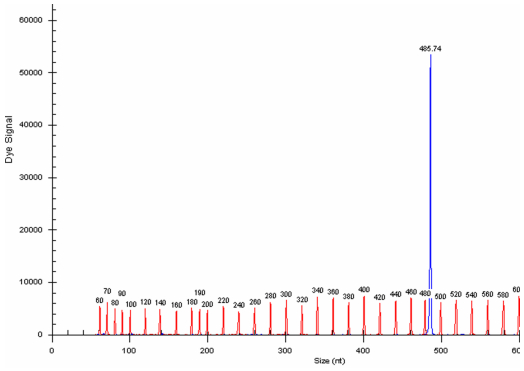
Supplementary figure 1



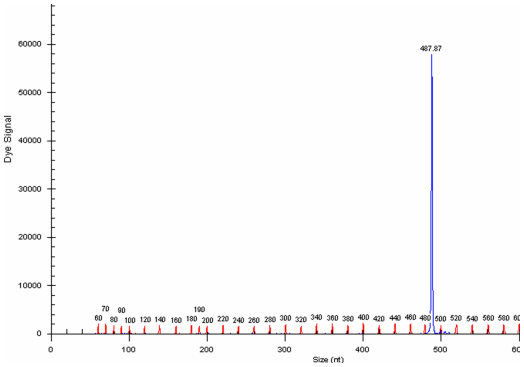
Supplementary Figure 1.

Complexity of T-cell Vβ receptor. Complete spectratype analysis of T-cell receptor Vβ complexity at the time of leukemia presentation (top panel) and after the patient received chemotherapy (bottom panel).

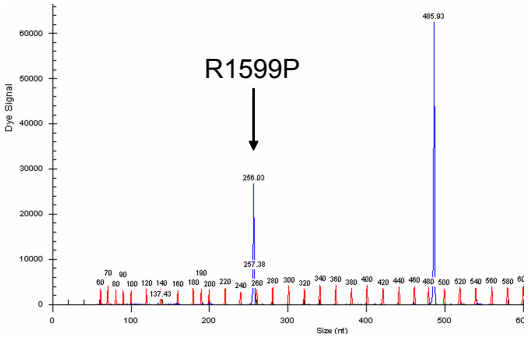
Supplementary figure 2



Wild type



Day 539



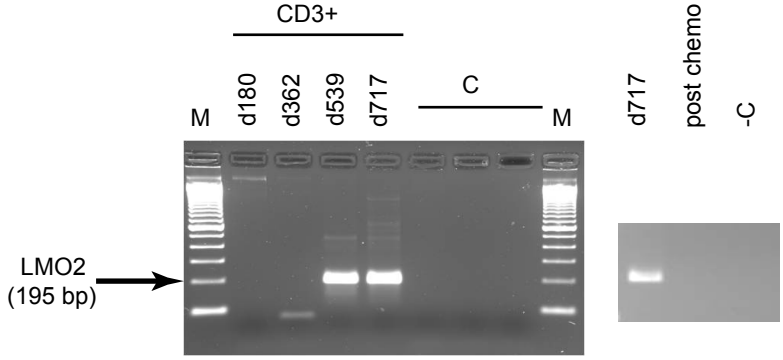
Day 717

Supplementary Figure 2. Detection of the *NOTCH1* mutation by amplification-refractory mutation-system (ARMS)

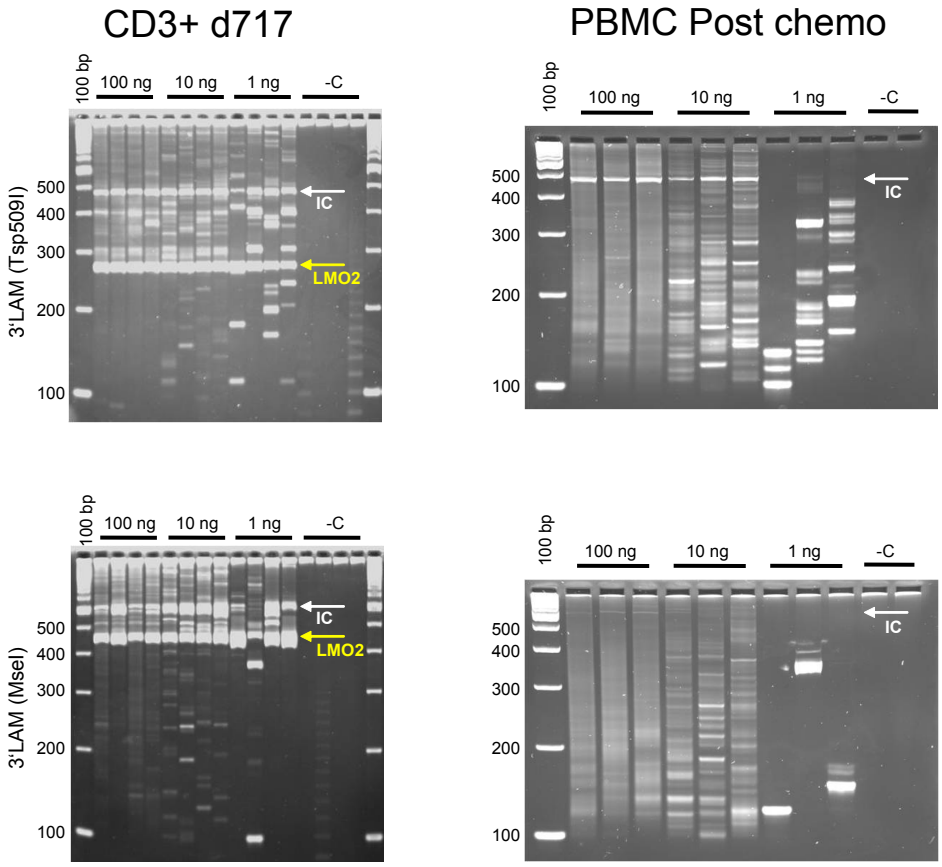
ARMS PCR analysis of NOTCH1 point mutation. The R1559P substitution in NOTCH1 was evident in leukemic cells, but not before (day 539 post-therapy) at the resolution of this assay (sensitive to approximately 1 mutated sequence in 1000).

Supplementary Figure 3

Supplementary Figure 3a



Supplementary Figure 3b



Supplementary Figure 3. Tracking of the clone containing an insertion upstream of *LMO2*

(a) Tracking of the malignant clone. Clone specific PCR was performed on T cell DNA derived from different time points after gene therapy (left panel). Leukemia was clinically apparent at 717 days after gene therapy, but the malignant clone could first be detected at 539 days after gene therapy (195 bp PCR product, sensitivity approximately less than 1 mutated sequence in 1000). The integration upstream of *LMO2* was not evident by this specific tracking PCR at day 861, 126 days after the patient received chemotherapy treatment (post-chemo, right panel). Numbers indicate days after gene therapy, M= size marker, C = negative control.
(b) Integration site clonality. Linear amplification-mediated PCR (LAM PCR) was performed on 100, 10 or 1 ng of CD3 leukemia DNA (day 717 after gene therapy, left panels) or on the same amounts of DNA from post chemotherapy PBMCs (day 861 after gene therapy, 126 days post-chemotherapy). LAM PCR was performed using either *Tsp509*I (top panels) or *Mse*I (lower panels). Leukemia samples show oligoclonality, with a major band at the size expected for the *LMO2* integration. This clone resolved after chemotherapy treatment, with a return to polyclonality (shown by the smear with 100ng of input DNA, or multiple bands with less DNA). There is no evidence of a clonal expansion after chemotherapy. 100 bp = size ladder, -c = negative control, IC= internal control.