# Proportions of blood-borne Vδ1+ and Vδ2+ T-cells are associated with overall survival of melanoma patients treated with ipilimumab

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# Supplemental material

**Table A1: Association with patients' OS of γδ T-cell subsets during/after treatment with ipilimumab**

| | Time points | Total n | Cut-off [freq.] | n | % | Pairwise comparison (p-value) | % dead | Median survival (months) | 1-year survival rate (95% CI) | | 2-year survival rate (95% CI) | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Vδ1+ cells** | Early vs. baseline | 14 | ➔/↑ | 7 | 50.0 | 0.625 | 100 | 7 | 14.3% | (0%; 40.2%) | 0 | | 0.441 |
| | | | ↓ | 7 | 50.0 | | 85.7 | 6 | 28.6% | (0%; 62.1%) | 14.3 | (0%; 40.2%) | |
| | Intermediate vs. baseline | 25 | ➔/↑ | 16 | 64.0 | 0.089 | 75.0 | 7 | 31.3% | (13.8%; 61.2%) | 25.0 | (3.8%; 46.2%) | 0.251 |
| | | | ↓ | 9 | 36.0 | | 55.6 | 21 | 66.7% | (35.9%; 97.5%) | 44.4 | (11.9%; 76.9%) | |
| | Late vs. baseline | 23 | ➔/↑ | 13 | 56.5 | 0.185 | 76.9 | 8 | 38.5% | (12.0%; 65.0%) | 23.1 | (0.2%; 46.0%) | 0.253 |
| | | | ↓ | 10 | 43.5 | | 60% | 11 | 50.0% | (19.0%; 81.0%) | 40 | (9.6%; 70.4%) | |
| **Vδ2+ cells** | Early vs. baseline | 17 | ➔/↑ | 7 | 50.0 | 0.292 | 100 | 6 | 14.3% | (0%; 40.2%) | n.r. | | 0.586 |
| | | | ↓ | 7 | 50.0 | | 85.7 | 7 | 28.6% | (0%; 62.1%) | 14.3 | (0%; 40.2%) | |
| | Intermediate vs. baseline | 24 | ➔/↑ | 6 | 25.0 | **0.001** | 33.3 | n.r. | 100% | | 66.7 | (29.1%; 100%) | **0.039** |
| | | | ↓ | 18 | 75.0 | | 77.8 | 7 | 33.3% | (11.5%; 55.1%) | 22.2 | (3.0%; 41.4%) | |
| | Late vs. baseline | 23 | ➔/↑ | 7 | 30.4 | **0.017** | 28.6 | n.r. | 85.7% | (59.8%; 100%) | 71.4 | 37.9%; 100%) | **0.007** |
| | | | ↓ | 16 | 69.6 | | 87.5 | 7 | 25.0% | (3.4%; 46.2%) | 12.5 | (0%; 28.8) | |

**Figure A1**

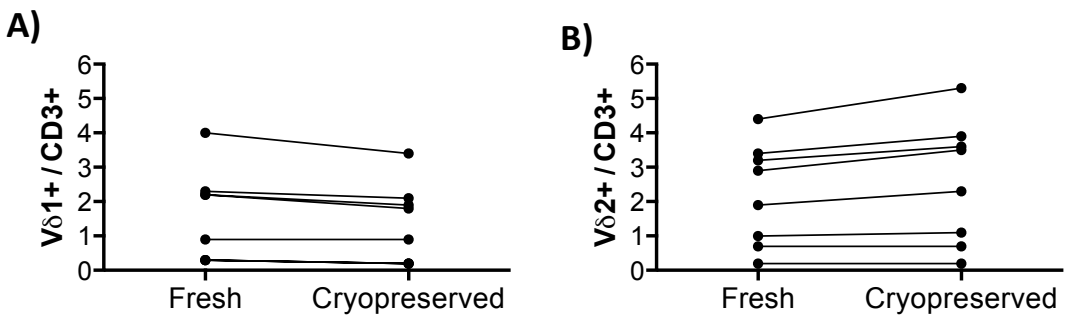


**Figure A1: Analysis of the impact of cryopreservation on the abundance of the major γδ T-cell subsets in PBMCs.** Frequencies of Vδ1+ cells among T-cells are displayed in panel A) and those of Vδ2+ cells in panel B). Paired analysis of 8 healthy donors using freshly isolated PBMCs (left) or thawed PBMCs after cryopreservation (right). Differences in frequencies were only marginal, justifying the use of cryopreserved PBMCs for γδ T-cell analysis. These experiments were performed in Singapore and approved by the National University of Singapore Institutional Review Board (IRB 10-445); an informed consent was obtained from all participants. A MACS-Quant flow cytometer and FlowJo 10.1.5 was used for this analysis.

**Figure A2**

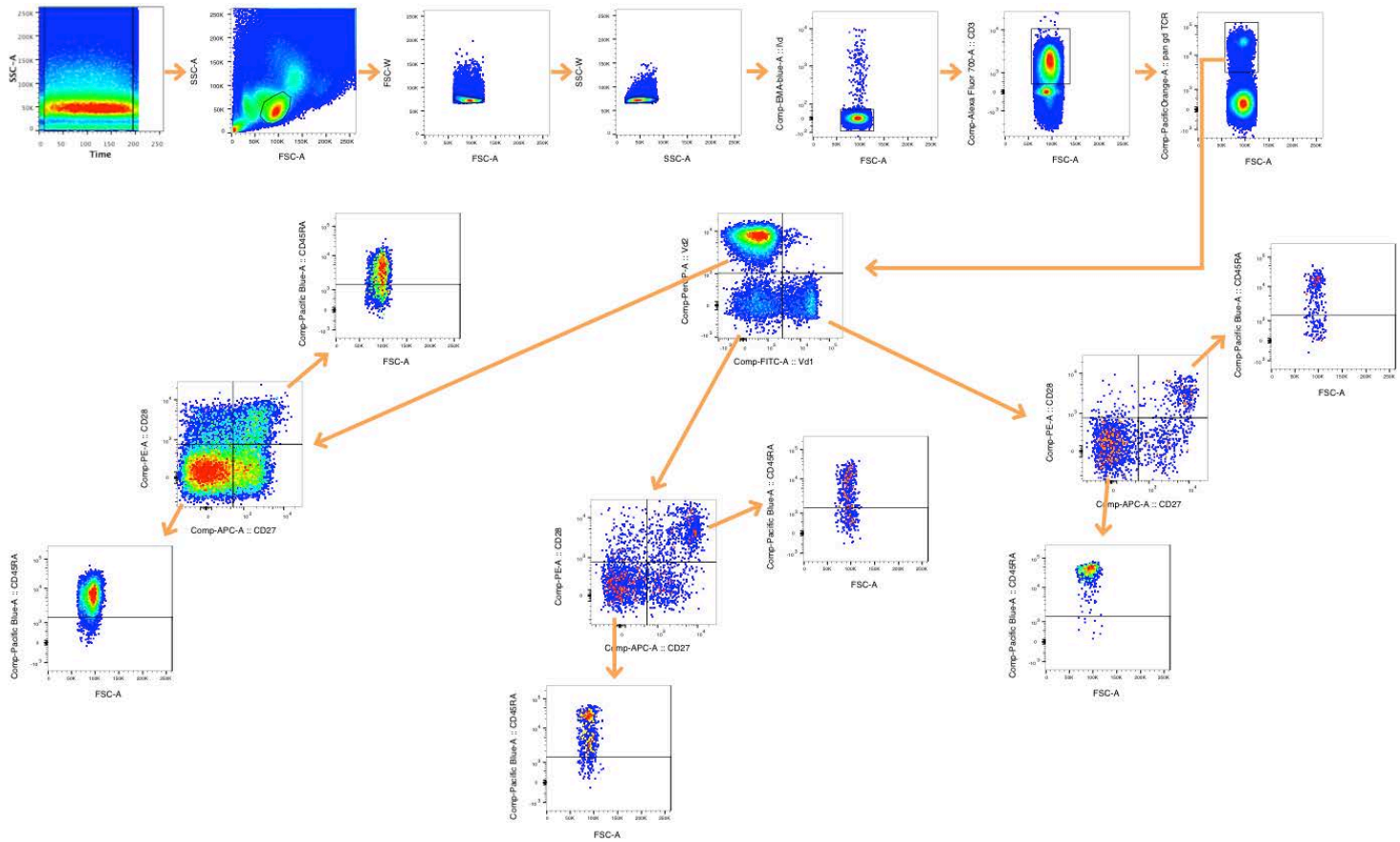


**Figure A2: Gating strategy for the quantification of γδ T-cell phenotypes.** The populations are displayed in FlowJo´s pseudo-color plot using large dots for a comprehensive visualization of small populations.

**Figure A3**

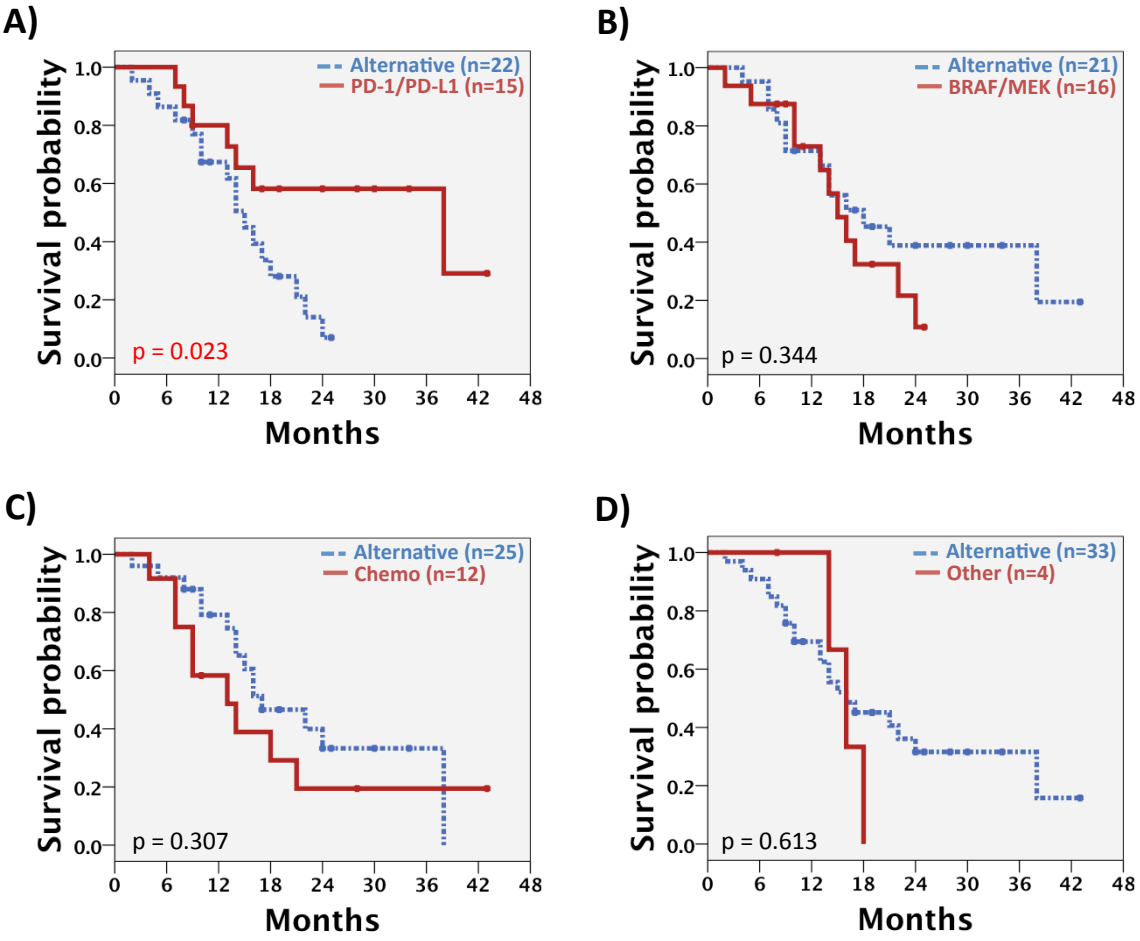


**Figure A3: Impact of subsequent treatments on OS.** Thirty-seven patients received subsequent treatments after the administration of ipilimumab. A) 15 patients subsequently received anti-PD-1/PD-L1 antibodies and had a survival benefit over the 22 that received an alternative subsequent treatment. OS was not different according to subsequent treatment with BRAF/MEK inhibitor B), chemotherapy C), or other therapies D).

**Figure A4**

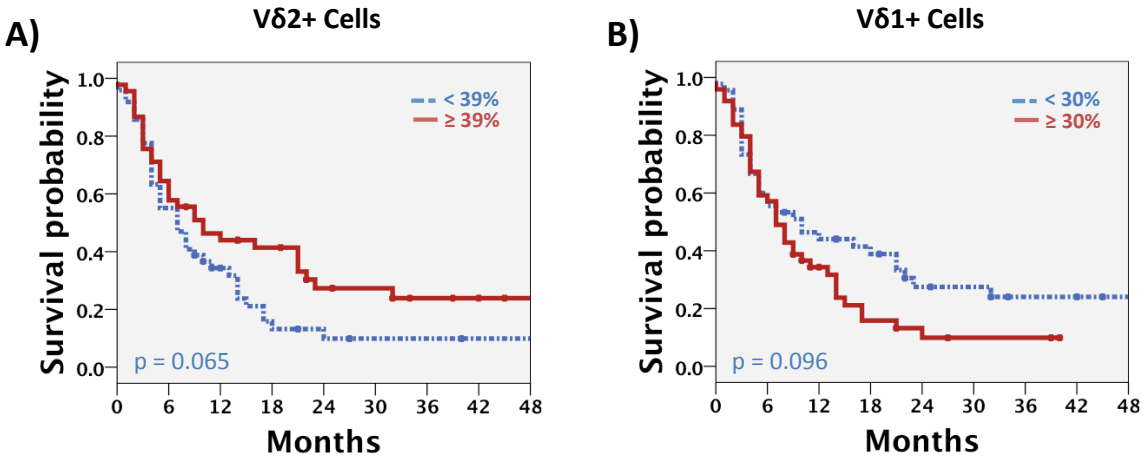


**Figure A4**: **Association of Vδ1+ and Vδ2+ cells with OS after exclusion of patients that received subsequent PD-1/PD-L1 treatment.** OS according to frequencies of Vδ2+ A) and Vδ1+ cells B). The separation of curves was similarly marked for both compartments compared to the analysis of the entire cohort. However, the p-values only indicated a trend for difference, presumably because of the reduced sample size.

**Figure A5**

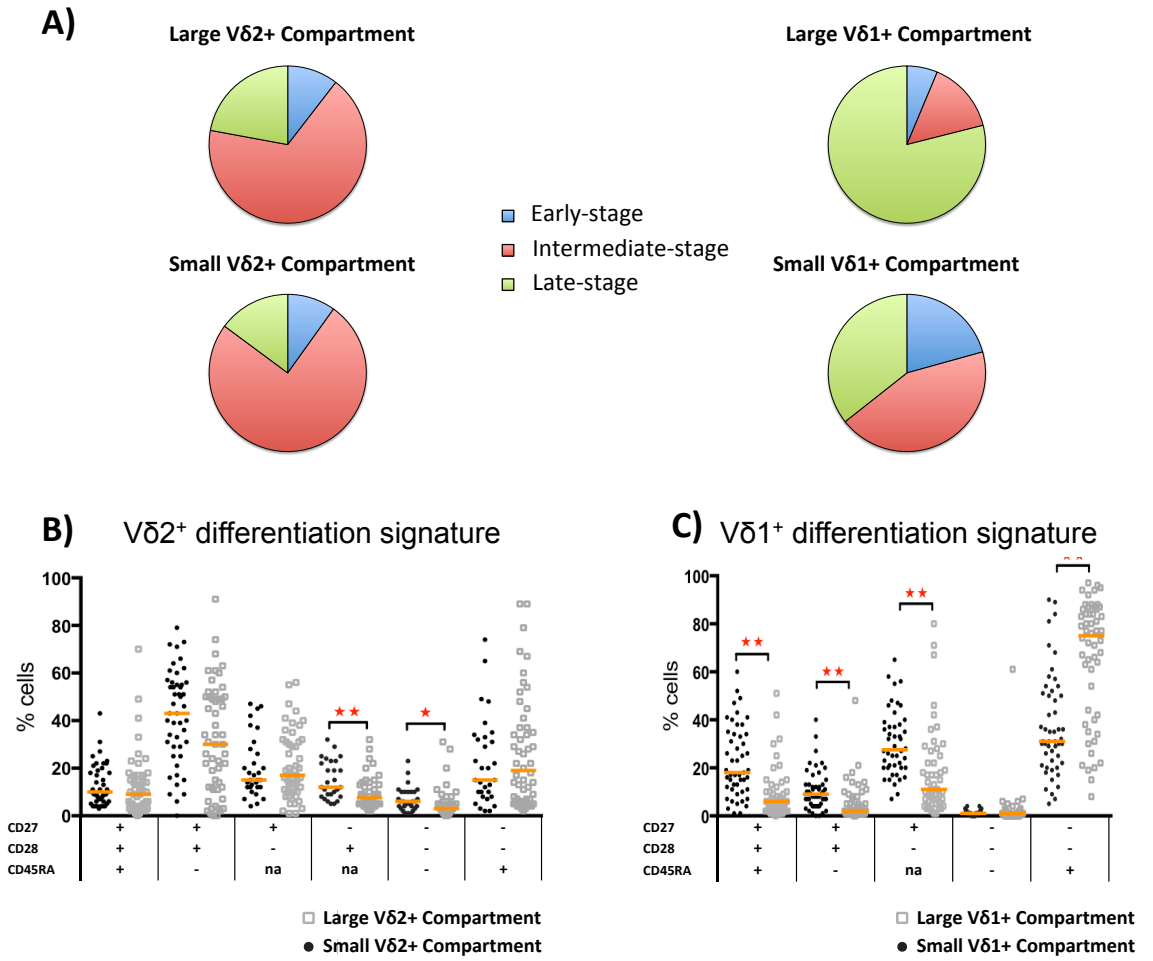


**Figure A5: Differentiation signatures of Vδ1+ and Vδ2+ subsets before starting ipilimumab.** As associations with prognosis were observed for the frequencies of Vδ1+ or Vδ2+ cells, the analysis was performed separately for patients with larger vs. smaller Vδ1+ or Vδ2+ subsets. **A)** Proportions of differentiation phenotypes in the Vδ1+ and Vδ2+ subsets. Differentiation signatures at single patient levels for the Vδ2+ subset in **B)** and the Vδ1+ subset in **C)**. Statistical evaluation was performed with the Mann Whitney U test. Median is indicated for each group. Annotation: * p ≤0.001 ; ** p ≤0.0001

**Figure A6**

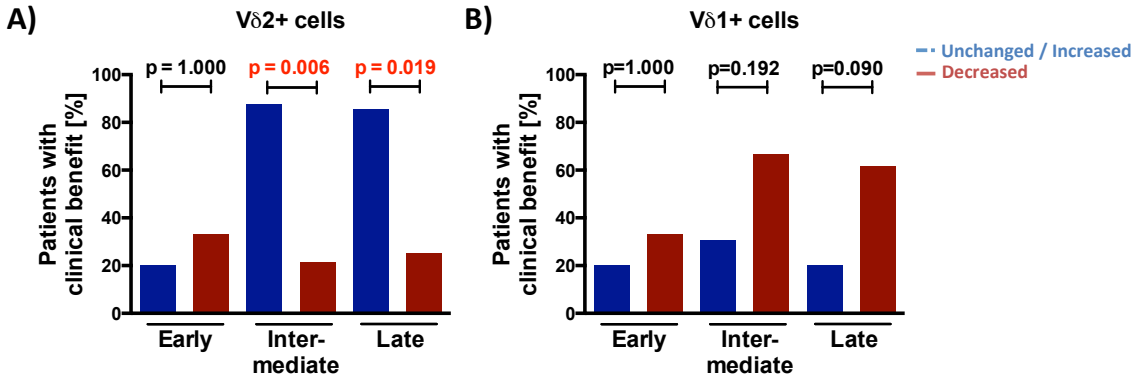


**Figure A6: Association of clinical benefit (irRC) with changes in abundance of Vδ2+ and Vδ1+ compartments during administration of ipilimumab**. The proportions of patients experiencing a clinical benefit (CR, PR or SD) according to changes of Vδ2+ A) or Vδ1+ cells B). Changes were grouped into “decreased” or “unchanged/increased” comparing the frequency at one of the three analyzed later time-points (TP) relative to the baseline value. The analysis of alterations in the Vδ2+ compartment comprised 17 (early TP), 24 (intermediate TP), 23 (late TP) patients. The analysis of changes in the Vδ1+ subset included 14, 25, 23 patients, respectively.

**Figure A7**

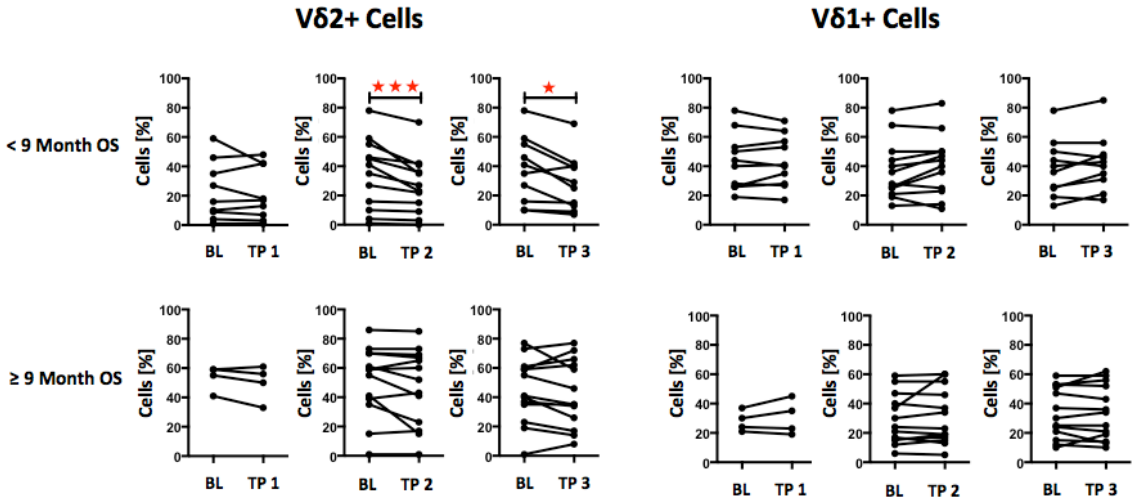


**Figure A7: Alterations in Vδ1+ and Vδ2+ cell frequencies after starting ipilimumab according to prognosis.** Wilcoxon matched pair test identified significant decreases in frequencies of Vδ2+ cells for TP2 and TP3 compared to BL. Patients were further stratified into two balanced prognostic groups based on the median OS after starting ipilimumab (9 months) and are presented separately. Frequencies as analyzed after starting ipilimumab but before day 21 (TP 1), between days 22 and 42 (TP 2) and after day 42 (TP 3) were compared to baseline findings (BL). One patient with a follow-up time of less than 9 months was not considered. For statistical analysis the Wilcoxon matched-pairs signed rank test was applied. Annotation: * p≤0.05 ; ** p≤0.005