

Supplementary Figure 1. Schematic treatment schedule and CONSORT flow

chart

(a) Schematic treatment schedule. (b) CONSORT flow chart.



Supplementary Figure 2. Prophylactic vaccine maturation cocktail with Influvac compared to with Act-HIB

Monocyte-derived DC were matured for 48h with the conventional cytokine cocktail (cDC; II-1 β , IL-6, TNF α , PGE₂), or with either prophylactic vaccine cocktail BTI (BCG, Typhim, Influvac) or cocktail BTA (BCG, Typhim, Act-HIB) with PGE₂. (a) The expression of maturation markers CD80, CD83, CD86, and CCR7 was measured by flow cytometry. Results are shown as percentage of positive cells. The figures show mean \pm SEM of three experiments with different donors. (b) IL-12p70 production was measured by ELISA in the supernatant of DC cultures 48h after maturation. Per condition each symbol represents one donor. Means are shown for each maturation cocktail. (c) The allostimulatory capacity of the DC was tested in a mixed lymphocyte reaction. Allogeneic peripheral blood leukocytes were co-cultured with differently matured DC and T cell proliferation was measured by incorporation of tritiated thymidine. The graph represents mean \pm SEM counts per minute relative to cDC of three experiments with different donors, performed in triplicate. (d) DC were electroporated with mRNA encoding gp100. After 3h, gp100 protein expression was determined by FACS analysis. Filled curves show staining with specific antibody; thin-lined curves show the isotype control. Numbers indicate mean fluorescence intensity.



Supplementary Figure 3. Vaccine characteristics of first cycle of all patients (a) Expression of HLA-ABC, HLA-DR/DP, HLA-DQ, CD80, CD83, CD86, and CCR7 was analyzed by flow cytometry. (b) Tumor antigen expression by VAC-DC 2-4h after electroporation with mRNA encoding gp100 and tyrosinase. Data are shown as percentage of positive VAC-DC used for the first vaccination. The graphs represent mean ± SD of all patients. Data is shown separately for each administration route; intravenous/intradermal (i.v./i.d.) and intranodal (i.n.) injection of VAC-DC.



Supplementary Figure 4. Injection site reactions

(a) Example of injection site reaction (patient A-11) after intradermal injection with VAC-DC. (b) Induration of the injection site after regular flu vaccination (patient A-3). Showing redness over an area with a diameter of 3 to 4 cm. (c) Example of injection site reaction (patient B-12) after intranodal injection with VAC-DC, showing substantial swelling of the lymph node (region), redness of the overlying skin and discharge of pus. (d) [¹⁸F]FLT-PET/CT scan of patient B-12 showing profound lymphadenopathy of the right inguinal lymph nodes after intradermal injection with VAC-DC, with moderately increased [¹⁸F]FLT accumulation in the rim and absent tracer accumulation in the centre, suggestive of necrosis (arrow).