nature cancer

Article

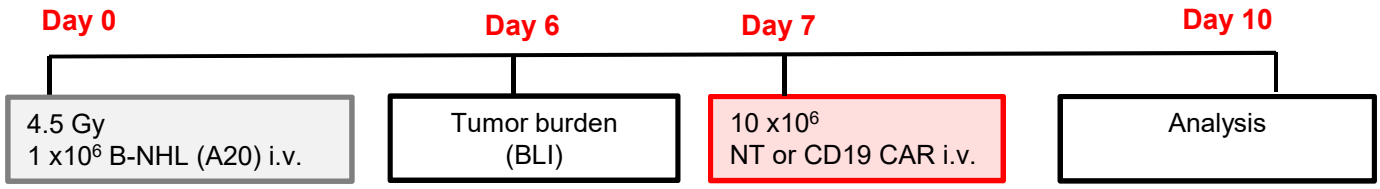
https://doi.org/10.1038/s43018-024-00764-7

Targeting TGFβ-activated kinase-1 activation in microglia reduces CAR T immune effector cell-associated neurotoxicity syndrome

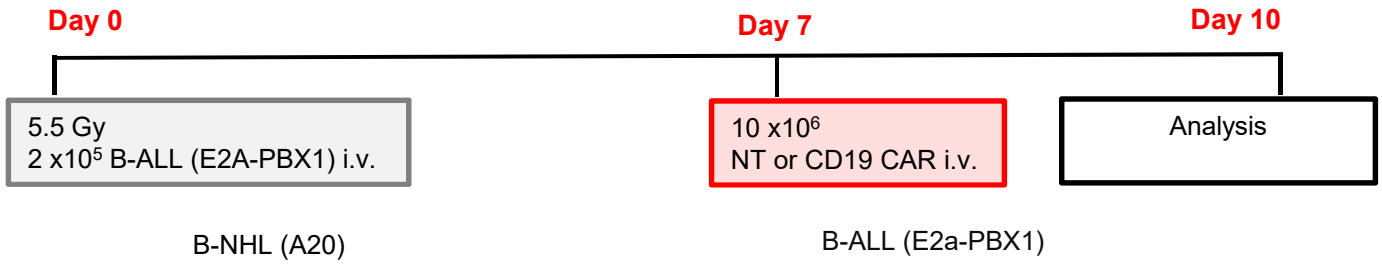
In the format provided by the authors and unedited

Suppl. Figure 1

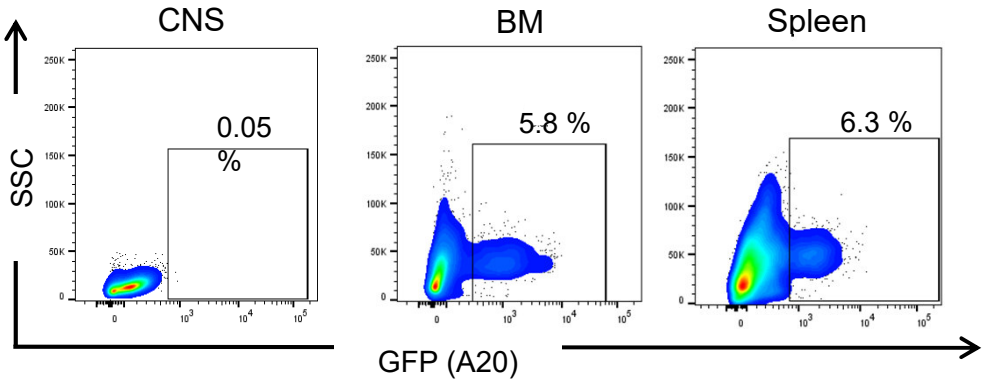
a BALB/c mice, A20 lymphoma



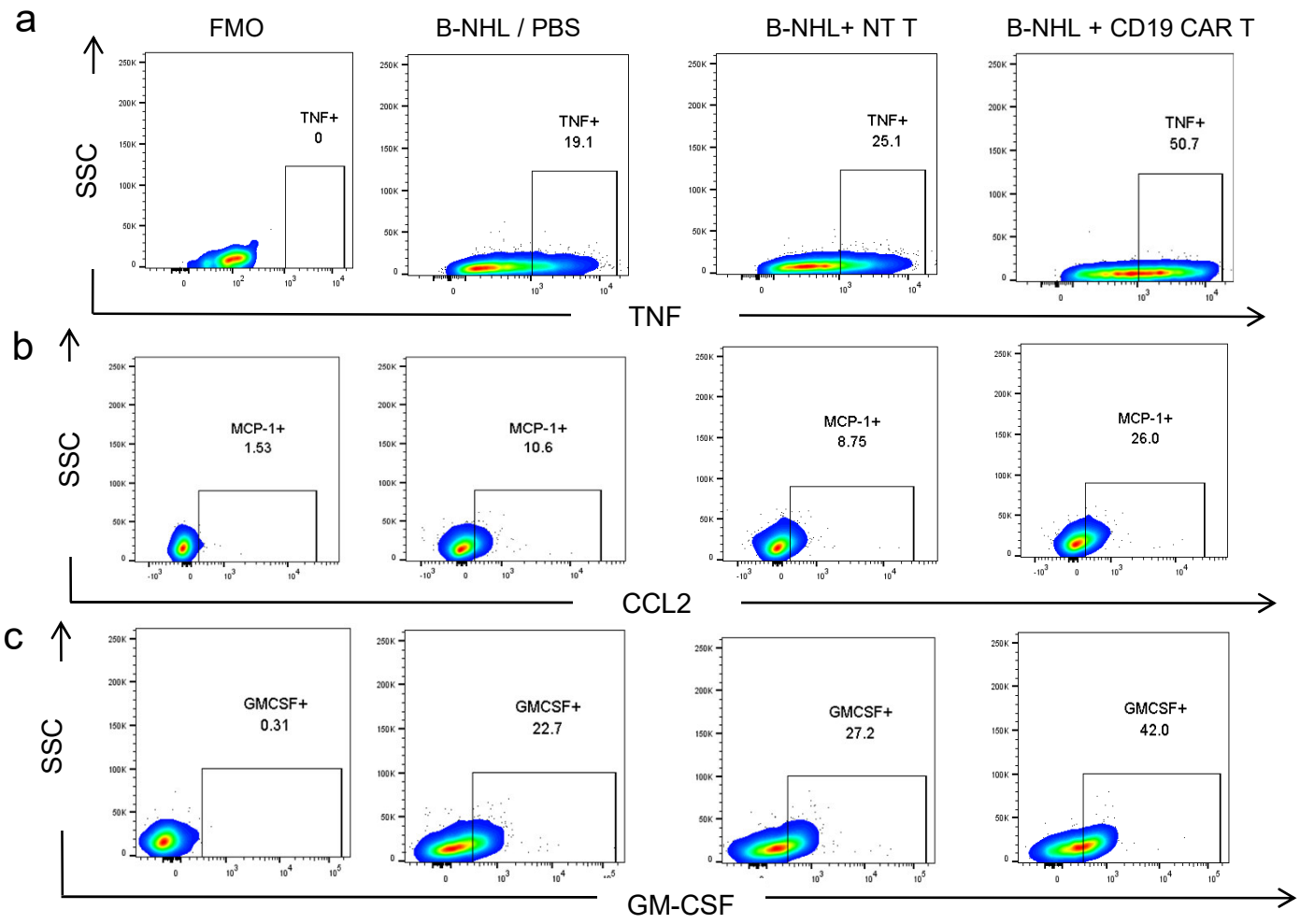
b C57Bl/6 mice, E2A-PBX1 leukemia



e

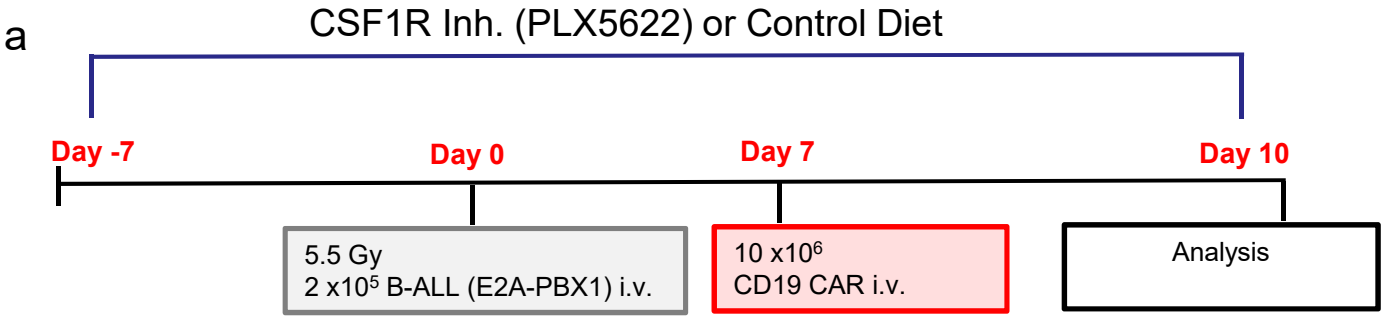


Suppl. Figure 2



Suppl. Figure 3

a



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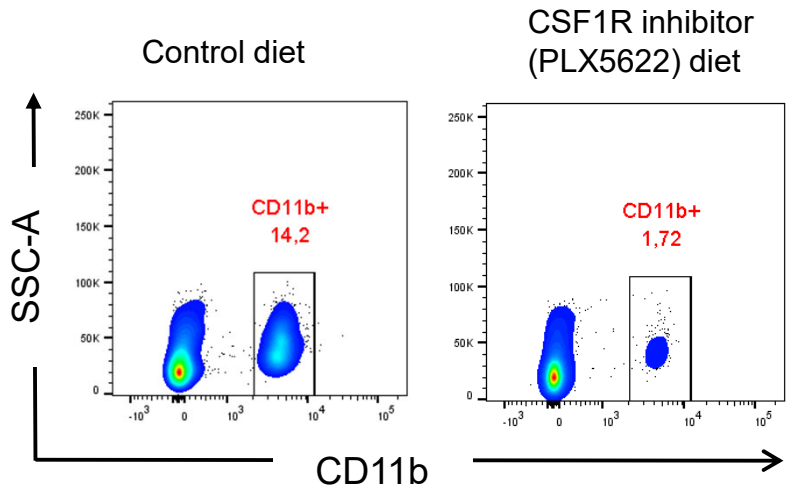

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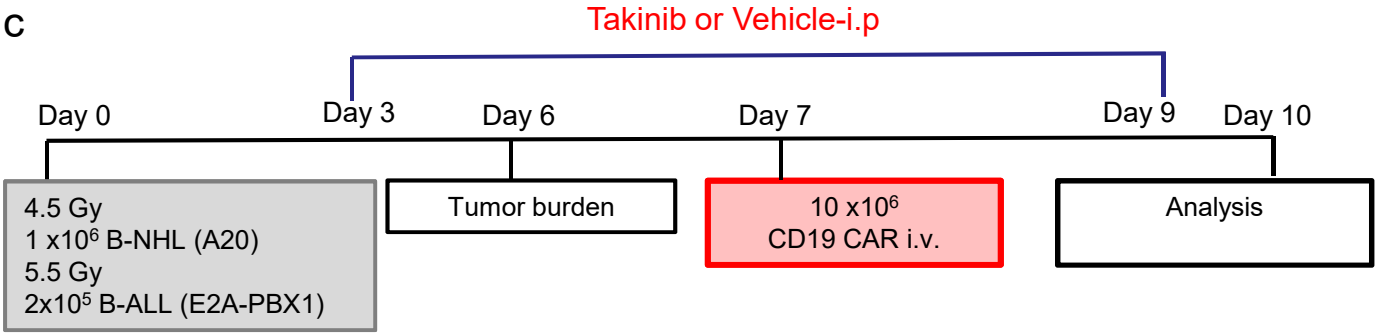

Legends for Suppl. figures

Suppl. Fig. S1: Treatment schedules for B-NHL and B-ALL ICANS models, representative flow cytometry plots showing gating for B-NHL cells in the brain, bone marrow and spleen
Suppl. Fig. S2: Flow cytometry plots for cytokine expression in microglia in B-NHL bearing mice
Suppl. Fig. S3: Treatment schedules for microglia depletion and Takinib treatment

Supplementary Figure legends

Suppl. Figure S1: Treatment schedules for B-NHL and B-ALL ICANS models, flow cytometry plots for B-NHL cells in brain, bone marrow and spleen

(a) A schematic representation of the ICANS mouse model using B-NHL cells (A20 Luc$^+$ *GFP*$^+$). BALB/c mice were sublethally irradiated with 4.5Gy and transplanted with 1 x 10^6 A20 Luc$^+$ *GFP*$^+$ cells. On day 6, engraftment was quantified with bioluminescence imaging (BLI). On Day 7, 10 x 10^6 CD19-CAR-T-cells were injected intravenously. Mice were sacrificed for analysis on day 10.
(b) A schematic representation of the ICANS mouse model using B-ALL cells. C57BL/6 mice were sublethally irradiated with 5.5 Gy and transplanted with 2 x 10^5 E2a-PBX1 cells. On day 7, 10 x 10^6 CD19-CAR-T-cells were injected intravenously. Mice were sacrificed for analysis on day 10.
(c) Representative flow cytometry plots indicating the proportion of *GFP*$^+$ A20 B cell lymphoma cells in the CNS, BM, and spleen.

Suppl. Figure S2: Cytokines on the protein level in the CNS of mice without tumor after NT T cell or CD19 CAR T cell transfer
(a) The scatter plot shows the quantification of TNF (pg/ml) of the homogenized CNS of tumor free BALB/c mice, treated with NT (mice, n=6) or CD19-CAR (mice, n=6) T-cells, 72h after T-cell transfer. The experiment was performed once (mean ± s.e.m.). Each data point represents an individual mouse. *P*-values were calculated using two-tailed student's unpaired *t*-test.
(b) The scatter plot shows the quantification of IL-6 (pg/ml) of the homogenized CNS of tumor free BALB/c mice, treated with NT (mice, n=6) or CD19-CAR (mice, n=6) T-cells, 72h after T cell transfer. The experiment was performed once (mean ± s.e.m.). Each data point represents an individual mouse. *P*-values were calculated using Mann-Whitney test.
(c) The scatter plot shows the quantification of CCL2 (pg/ml) of the homogenized CNS of tumor free BALB/c mice, treated with NT (mice, n=6) or CD19-CAR (mice, n=6) T-cells, 72h after T cell transfer. The experiment was performed once (mean ± s.e.m.). Each data point represents an individual mouse. *P*-values were calculated using two-tailed student's unpaired *t*-test.

Supp. Figure S3: Treatment schedules for microglia depletion and Takinib treatments
(a) Schematic representation of CSF1R inhibitor treatment. Mice were maintained on CSF1R inhibitor (PLX5622) diet or control diet from day -7 until day 10. Behaviour studies were performed on day 10.
(b) Representative flow cytometry plots showing CD11b-positive cells from the CNS of B-ALL-bearing mice maintained on either a control diet or CSF1R inhibitor (PLX5622) diet.
(c) Treatment sketch for takinib treatments with the B-NHL and B-ALL ICANS mouse models. Mice were treated as per the schedule (Supp. Fig 1a, b). Mice were treated intra-peritoneal with takinib from day 3 until day 9.