Supplementary Figure legends

Supplementary Figure 1. Short-term effects of BRAFi or oHSV on immune cell populations in an orthotopic immunocompetent mouse model of thyroid cancer.

Thyroid cancer cell line TBP-B79 was injected unilaterally into the left thyroid lobe of C57Bl/6 mice (n=4/group). After tumors were established, mice were treated daily with BRAF inhibitor (40 mg/kg) or vehicle control by oral gavage. Tumors were dissected 4 days post-start of treatment and tumor-infiltrating lymphocytes were analysed via flow cytometry. (A) BRAFi treatment and collection schedule for TBP-B79 tumor model. (B) Percentage of Ki67+ CD4eff, CD4reg or CD8+ cells. (C) Percentage of GzmB+ CD4eff, CD4reg or CD8+ cells. (D) Thyroid cancer cell line TBP-B79 was injected unilaterally into the left thyroid lobe of C57Bl/6 mice (n=4-6 / group). After tumors were established, mice were treated with a single injection of oHSV (5x10^5 pfu) or vehicle control by intratumoral injection. Tumors were dissected 48h post-virus infection and tumor-infiltrating lymphocytes were analysed by flow cytometry. Treatment and collection schedule for TBP-B79 tumor model. (E) Percentage of Ki67+ CD4eff, CD4reg or CD8+ cells. (F) Percentage of GzmB+ CD4eff, CD4reg or CD8+ cells. Each dot represents an individual mouse. Statistical analysis: unpaired nonparametric Mann-Whitney tests were performed using Prism Software (GraphPad). Comparison with p-values over 0.05 are deemed not statistically significant. BRAFi, BRAF inhibitor; ns, not significant; oHSV, oncolytic herpes simplex virus; LN, lymph node.

Supplementary Figure 2. Combined treatment with oHSV / BRAFi activates T-cells and increases expression of PD-L1, PD-L2 and CTLA-4 in the tumors.

(A) C57Bl/6 mice (n=3-4 / group) were subcutaneously implanted with the murine thyroid cancer cell line TBP-B79 in the right flank (3x10^6c/mouse) and mice were treated with BRAFi (40 mg/kg daily by oral gavage) and oHSV (5x10^5 pfu x 3 injections by intratumoral injection) in combination or vehicle controls. On treatment day 11 tumors were dissected, total RNA isolated and gene expression analysis was performed using the murine PanCancer Immune Profiling Panel from NanoString Technologies. (B) z-scores of differentially expressed (DE) genes with a log_2-fold change >1 and adjusted P <0.1) between vehicle-treated (n=3) and oHSV / BRAFi-treated (n=4) presented in a heat map. BRAFi, BRAF inhibitor; oHSV, oncolytic herpes simplex virus; DE, differentially expressed.

Supplementary Figure 3: Confirmation of flow cytometry analysis of tumors and tumor-draining lymph nodes in the TBP-67 model.

C57Bl/6 mice (n=6 / group) were subcutaneously implanted with the murine thyroid cancer cell line TBP-67 in the right flank (3x10^6c/mouse) and mice were treated with BRAFi (40 mg/kg daily by oral gavage) and oHSV (5x10^5 pfu x 3 injections by intratumoral injection) as single agents or in combination as detailed in Figure 5A. On treatment day 11, tumors were dissected and tumor-infiltrating lymphocytes (TILs) and tumor-draining lymph nodes were analysed via flow cytometry. (A) Ratio of tumor-infiltrating CD8+/CD4reg. (B) Percentage of Ki67+ CD4eff, CD4reg or CD8+ cells. (C) Percentage of GzmB+ CD4eff, CD4reg or CD8+ cells in tumors. (D) Percentage of PD-1+ tumor-infiltrating lymphocytes (TILs): CD4eff, CD4reg and

CD8+T cells. (E) Percentage of CTLA-4+ tumor-infiltrating lymphocytes (TILs): CD4eff, CD4reg and CD8+ cells. (F) Percentage of Ki67+ lymphocytes in tumor-draining lymph nodes: CD4eff, CD4reg and CD8+T cells. (G) Percentage of GzmB+ lymphocytes in tumor-draining lymph nodes: CD4eff, CD4reg and CD8+T cells. (H) Percentage of PD-1+ CD4eff, CD4reg or CD8+ cells in tumor-draining lymph nodes. (I) Percentage of CTLA-4+ CD4eff, CD4reg or CD8+ cells in tumor-draining lymph nodes. Each dot represents an individual mouse. Statistical analysis: non-parametric Kruskal-Wallis test / Dunn's multiple comparisons were performed using Prism Software (GraphPad). Comparison with p-values over 0.05 are deemed not statistically significant. Teff (CD4+ / FoxP3-) and Treg (CD4+ / FoxP3+). BRAFi, BRAF inhibitor; ns, not significant; oHSV, oncolytic herpes simplex virus; LN, lymph node.

Supplementary Figure 4. Therapeutic efficacy of single agent treatments.

(A) C57Bl/6 mice (n=6/group) were subcutaneously implanted with murine thyroid cancer cell line TBP-B79 in the right flank (3x10^6c/mouse) and mice were treated with BRAFi (40mg/kg daily by oral gavage), oHSV (5x10^5 pfu x 3 by intratumoral injection) and anti-PD-1 (200 μg, intraperitoneal injection) or anti-CTLA-4 (150 μg, intraperitoneal injection) therapeutic antibodies or relevant controls as indicated. (B) Graphic display of tumor growth of single-agent treated mice. Tumor growth curves with data presented as mean +/- SEM. No significant differences were observed between groups. (C) Kaplan-Meier survival graph displaying the survival of the animals treated with different single agent therapies. Kaplan-Meier survival curves were compared using the log-rank (Mantel-Cox) test using Prism Software

(GraphPad). (D) Tumor growth curves displaying double and triple combination therapy groups with data presented as mean +/- SEM. (E) Kaplan-Meier survival graph displaying the survival of the animals treated in Supplementary Figure 2D). Kaplan-Meier survival curves were compared using the log-rank (Mantel-Cox) test using Prism Software (GraphPad). BRAFi, BRAF inhibitor; ns, not significant; oHSV, oncolytic herpes simplex virus; LN, lymph node.

Supplementary Table 1.

List of antibodies used in flow cytometry experiments.