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CLDN6-specific CAR-T cells plus amplifying RNA vaccine in relapsed or refractory solid tumors: the phase 1 BNT211-01 trial

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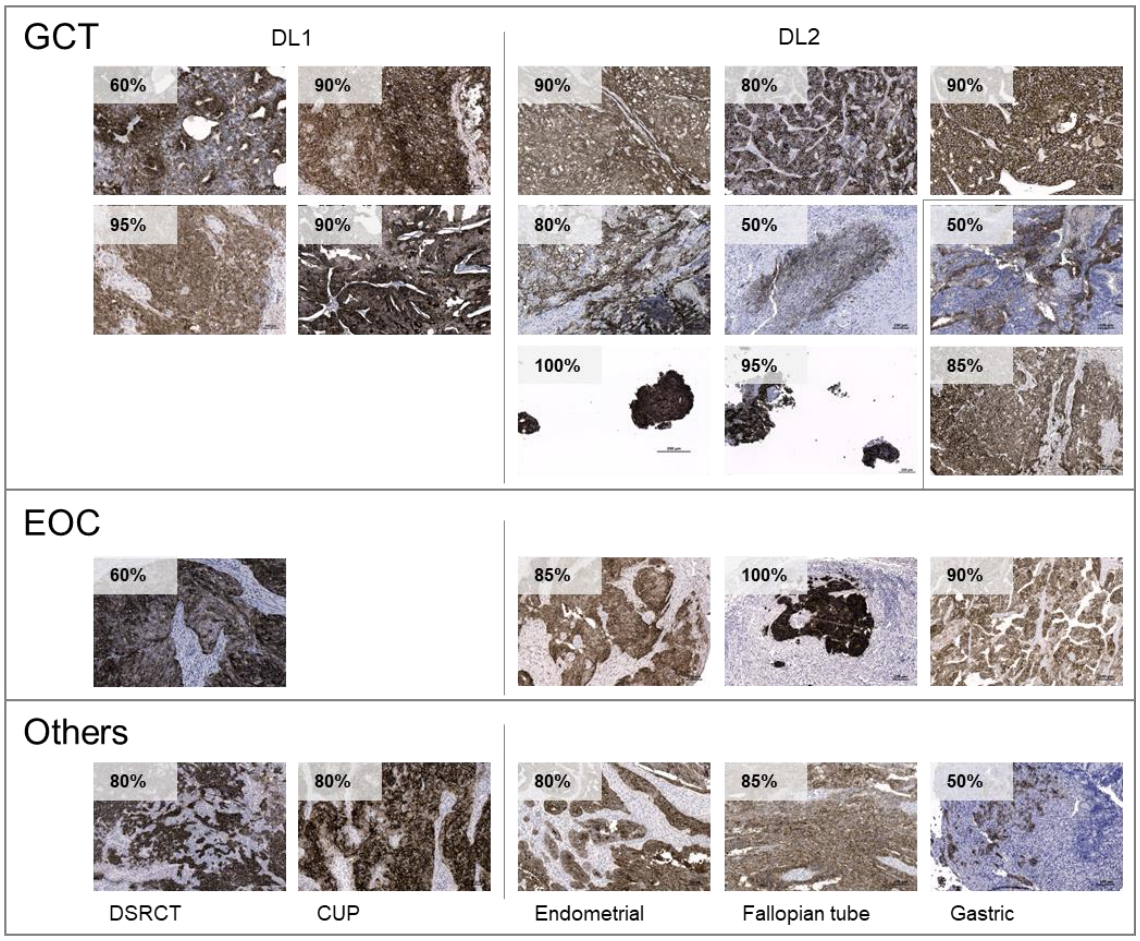
SUPPLEMENTARY INFORMATION

Supplementary Figure 1



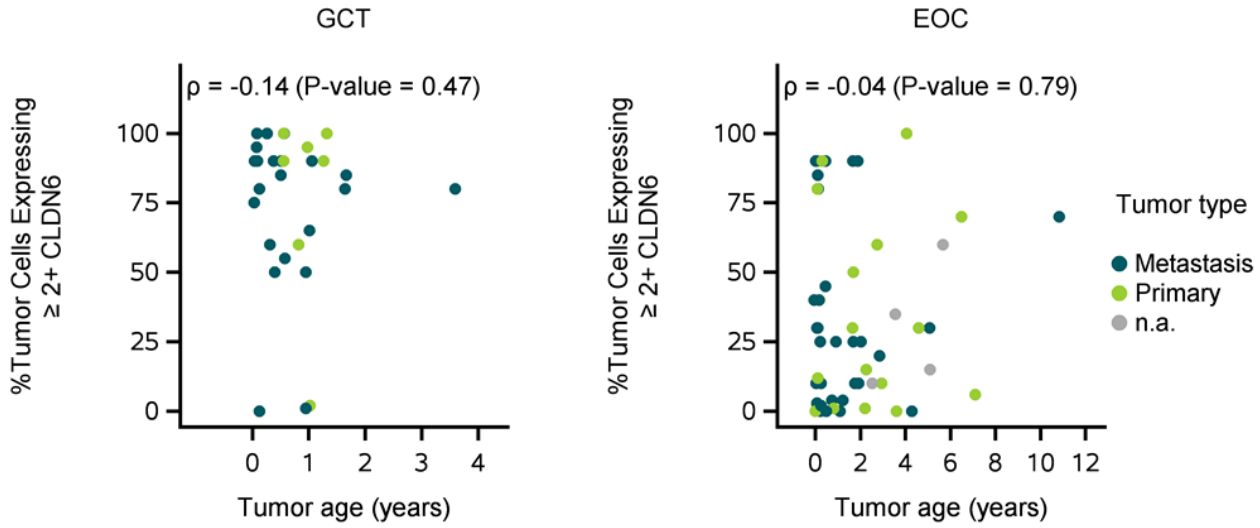
Supplementary Figure 1. CLDN6 pre-screening results of patients recruited to the trial.

Frequency of 2+/3+ CLDN6 expression revealed by immunohistochemical analysis of tumor tissue from patients treated with CLDN6 CAR-T cells grouped by indication and dose level. Cancers of "other" origin than GCT and EOC as indicated. Two patients at DL2 treated w/o LD are grouped.

Percentages indicate the number of tumor cells positive for intermediate (2+) or strong (3+) membrane expression of CLDN6. Immunohistochemical analysis was performed on two tumor samples per patient, with the stronger of the two values used to determine eligibility.

CLDN6, claudin 6; CUP, cancer of unknown primary; DSRCT, desmoplastic small round cell tumor; EOC, epithelial ovarian cancer; GCT, germ cell tumor; LD, lymphodepleting chemotherapy.

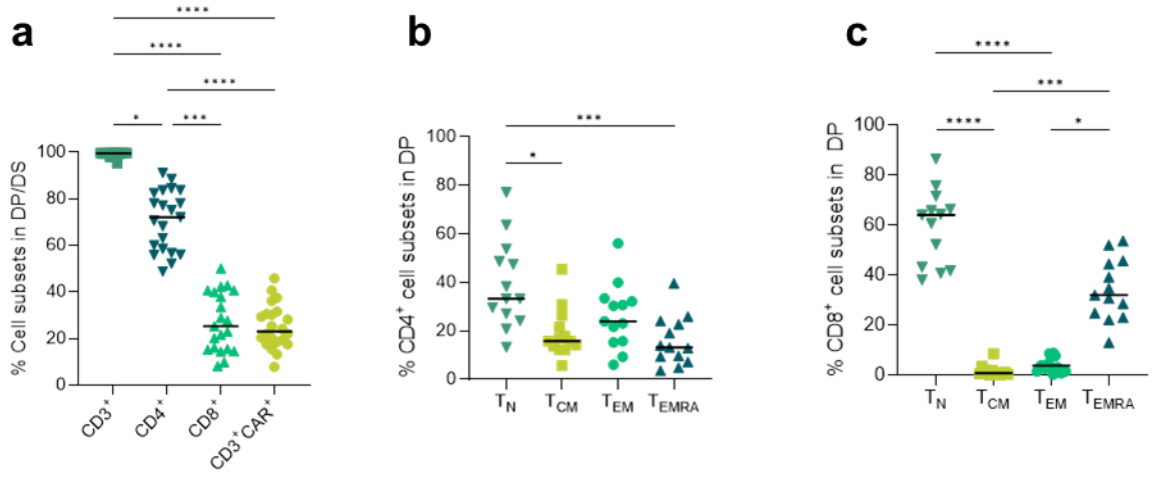
Supplementary Figure 2



Supplementary Figure 2: The intensity of CLDN6 expression by IHC staining in tumor samples does not correlate with the age of the analyzed tumor sample for both GCT and EOC patients.

Spearman rank correlation analysis of the age of pre-screening tumor samples and frequency of 2+/3+ CLDN6 expression in tumor cells revealed by immunohistochemical analysis from GCT and EOC patients. Tumor age refers to the time period between tissue collection and IHC analysis. Each dot represents a tumor sample: GCT patients, n=30; EOC patients, n=53. 2 patients with tumor collection dates not available were excluded from the analysis. Tumor age and CLDN6 expression were analyzed with Spearman's rank correlation coefficient.
n.a., information on tumor type not available; IHC, immunohistochemistry.

Supplementary Figure 3



Supplementary Figure 3: Characterization of the drug products by flow cytometry (CD4/CD8 distribution, CAR expression, and memory phenotype).

a, $CD3^+$, $CD4^+$, and $CD8^+$ cells were analyzed from bulk DP (n=21), $CD3^+CAR^+$ was analyzed from DS (n=22). **b**, **c**, TN, TCM, TEM, and TEMRA were analyzed in $CD4^+$ and $CD8^+$ T cells from bulk DP (n=13), respectively, based on sample availabilities. Horizontal line represents median frequency. Kruskal-Wallis test with Dunn's multiple comparison post hoc test was applied. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; **** $p \leq 0.0001$, with the exact adjusted P values as follows:

For %cell subsets in DP/DS:

Dunn's multiple comparisons test	**Summary**	**Adjusted P Value**
$CD3^+CAR^+$ vs. $CD3^+$	****	<0.0001
$CD3^+CAR^+$ vs. $CD4^+$	****	<0.0001
$CD3^+$ vs. $CD4^+$	*	0.0343
$CD3^+$ vs. $CD8^+$	****	<0.0001
$CD4^+$ vs. $CD8^+$	***	0.0002

For %CD4+ cell subsets in DP:

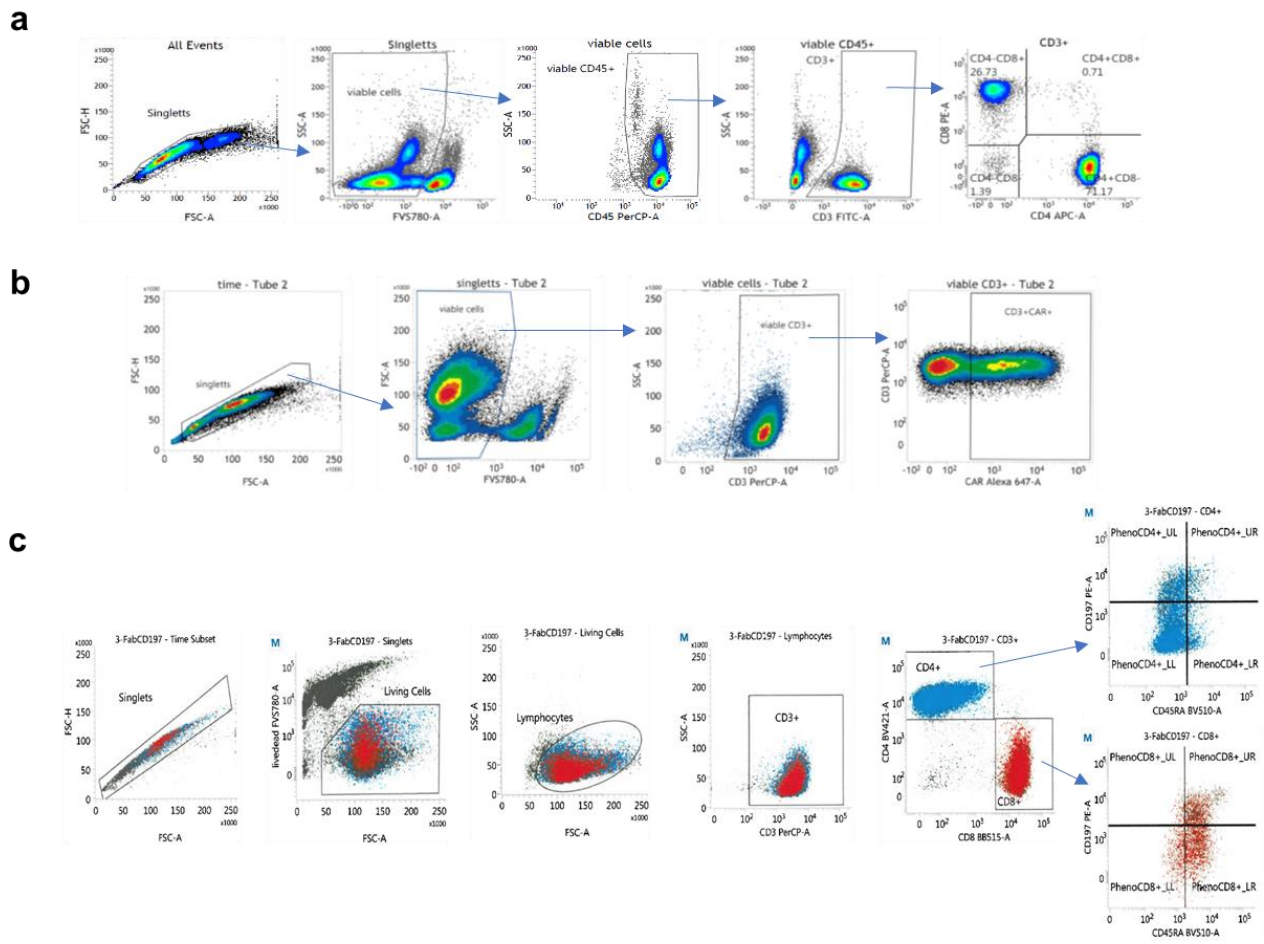
Dunn's multiple comparisons test	**Summary**	**Adjusted P Value**
T_{CM} vs. T_N	*	0.0157
T_N vs. T_{EMRA}	***	0.0010

For %CD8+ cell subsets in DP:

Dunn's multiple comparisons test	**Summary**	**Adjusted P Value**
T_{CM} vs. T_N	****	<0.0001
T_{CM} vs. T_{EMRA}	***	0.0002
T_N vs. T_{EM}	****	<0.0001
T_{EMRA} vs. T_{EM}	*	0.0264

CAR, Chimeric antigen receptor; DP, Drug Product; DS, Drug Substance; TN, naïve-like T cells; TCM, central memory T cells; TEM, effector memory T cells; TEMRA: effector memory re-expressing CD45RA T cells.

Supplementary Figure 4



Supplementary Figure 4: Gating strategies for immunophenotyping analysis.

a, Gating strategies to determine the percentage of $CD3^+$, $CD3^+CD4^+CD8^-$ cells, and CD3+CD4-CD8+ cells in bulk DP. **b**, Gating strategies to determine the percentage of $CD3^+CAR^+$ cells in DS. **c**, Gating strategies to determine the percentage of $CD45RA^+/CCR7^+$ (TN) cells, $CD45RA^-/CCR7^+$ (TCM) cells, $CD45RA^-/CCR7^-$ (TEM) cells, and $CD45RA^+/CCR7^-$ (TEMRA) cells in the $CD4^+$ and $CD8^+$ cell subsets in bulk DP.

DP, drug product; DS, drug substance; TN, naïve-like T cells; TCM, central memory T cells; TEM, effector memory T cells; TEMRA: effector memory re-expressing CD45RA T cells.