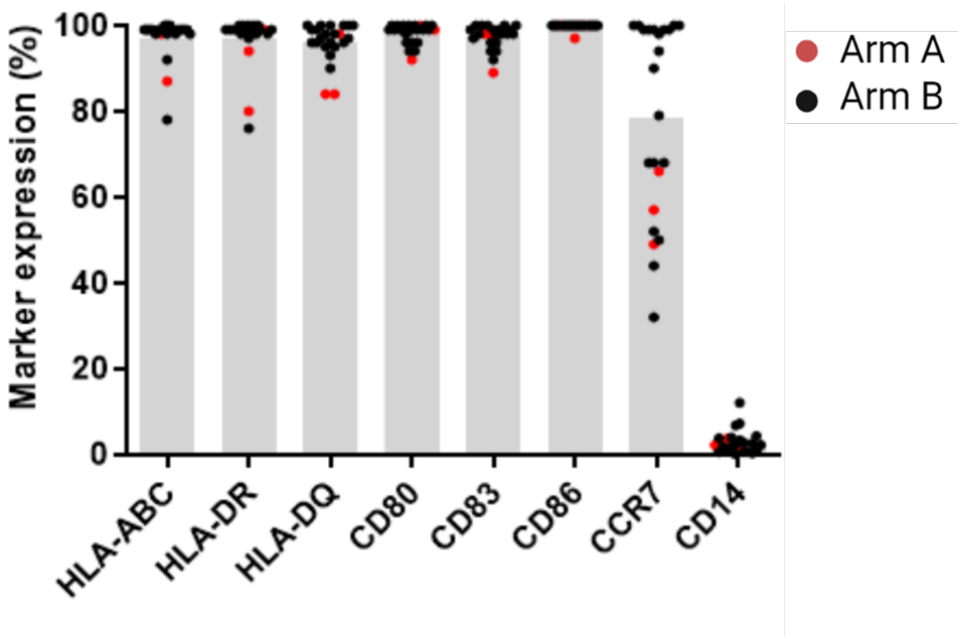
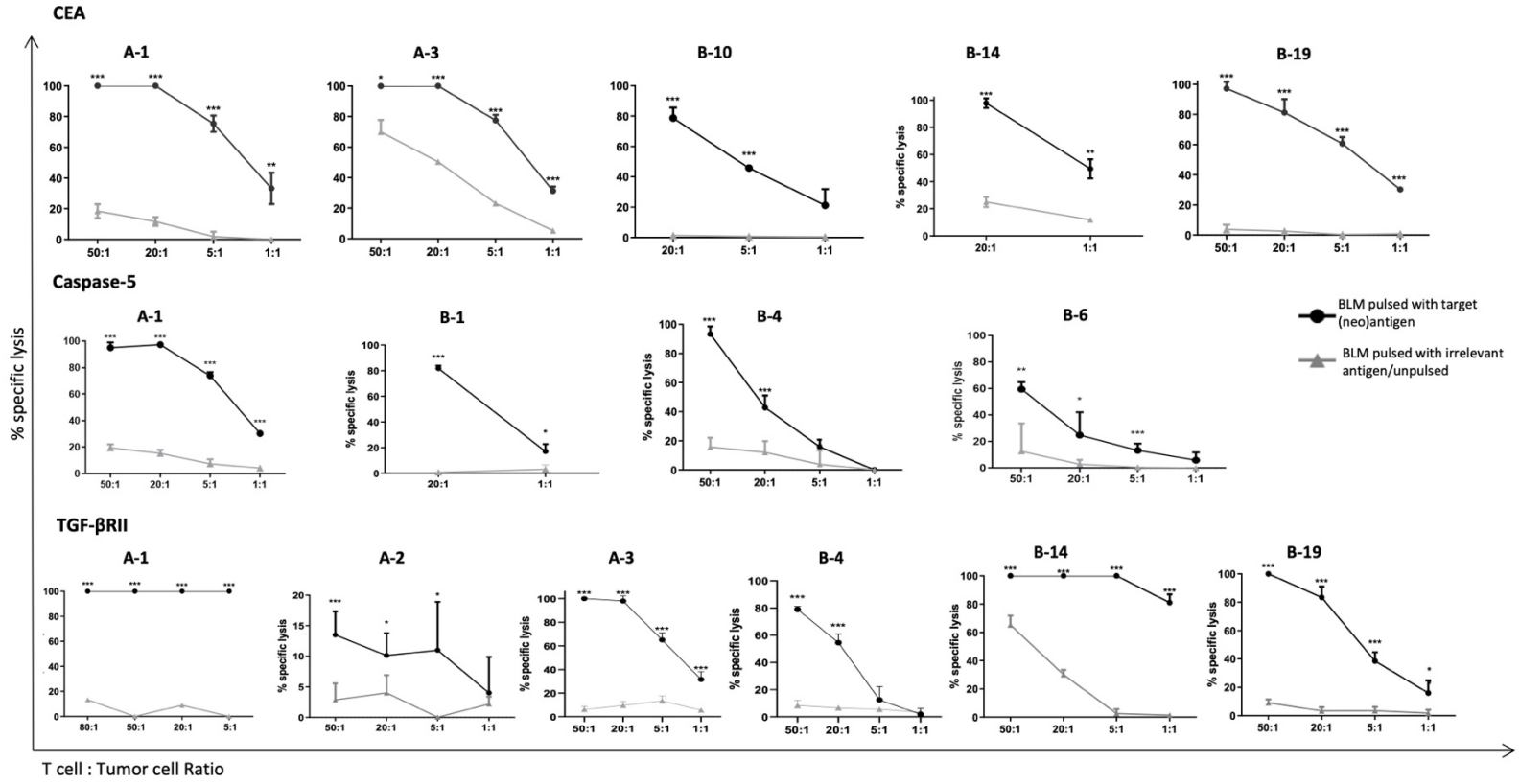
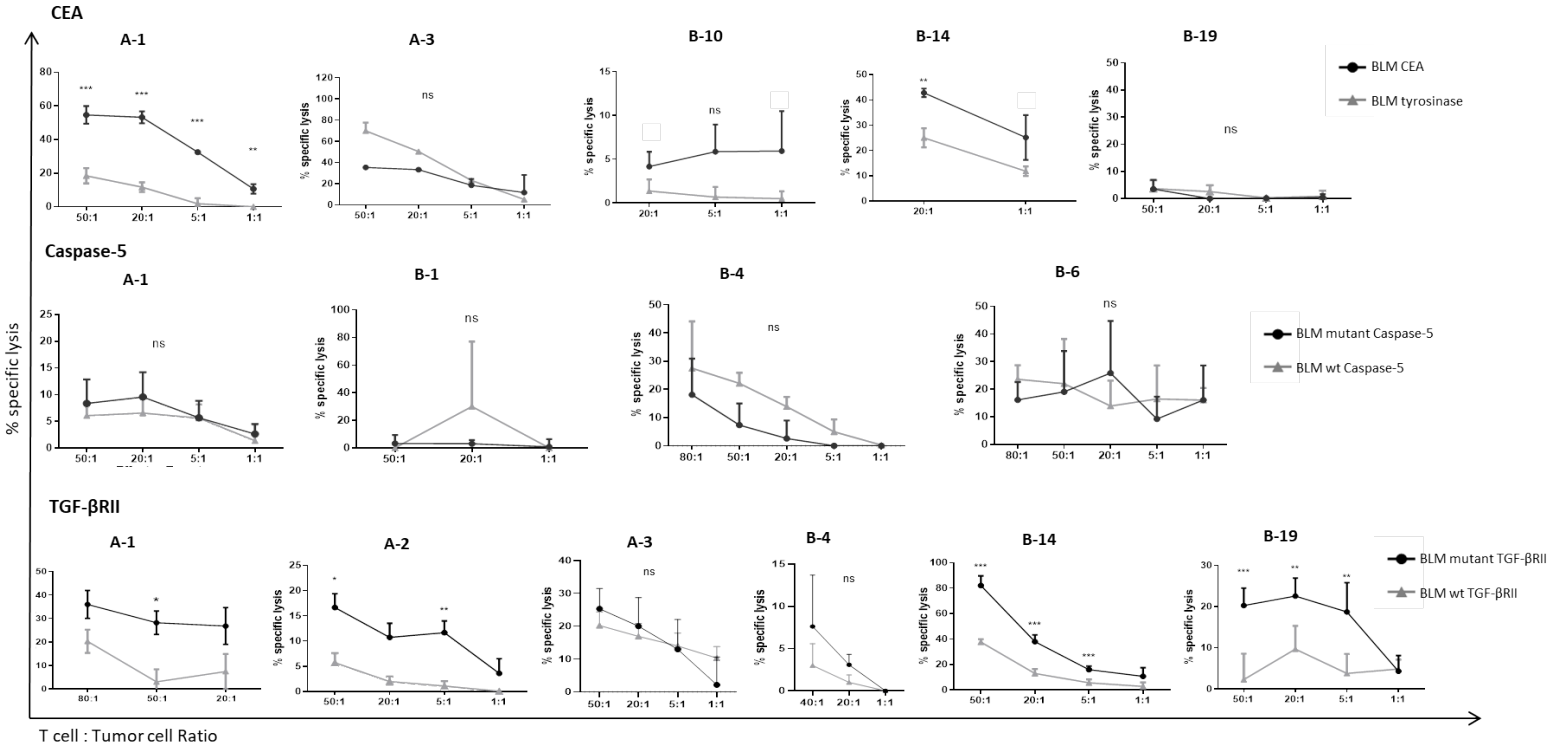
**Extended Data Fig. 1:** Phenotype of dendritic cell vaccines of all patients. Marker expression was analyzed by flow cytometry. Dots represent the result of a single patient. Lines represent the mean.



**Extended Data Fig. 2:** In-vitro lysis of tumor cells presenting (neo)antigen by patient CD8 T-cells. Target cells were labeled with calcein AM, loaded with HLA-A*02 binding peptides of CEA, caspase-5, or TGF-βRII and incubated with expanded (neo)antigen specific T-cells for 4 hours in different effector target ratios as shown. Specific lysis was calculated relative to maximum calcein release (Triton control) and is shown as mean ± SD from 3–6 replicate wells. Robust lysis was observed for CEA peptide-loaded tumor cells, with minimal or no lysis of unloaded cells or cells loaded with irrelevant gp100 peptide. Lysis was T cell-dependent across all patient samples tested. Specific lysis values >100% or <0% were capped at 100% and 0%, respectively, to account for technical variability. P-values less than ≤ 0.05 are indicated with *, ≤0.01 by ** and ≤0.001 by ***.



**Extended Data Fig. 3:** In-vitro lysis of tumor cells endogenously expressing the (neo)antigen or a control antigen by patient CD8 T-cells. Target cells endogenously expressing CEA or control antigen tyrosinase, mutant caspase-5 or as control wildtype caspase-5 and mutant TGF-βRII or wildtype TGF-βRII. All cell lines were labeled with calcein AM and incubated with expanded T-cells for 4 hours in different effector target ratios as shown. Specific lysis was calculated relative to maximum calcein release (Triton control) and is shown as mean ± SD from 3–6 replicate wells. Specific lysis of tumor cells endogenously expressing CEA was seen for 2/5 patients, caspase-5 for 0/4 patients and for TGF-βRII for 4/6 patients in a T-cell dependent manner. P-values less than ≤ 0.05 are indicated with *, ≤0.01 by ** and ≤0.001 by ***, ns: non-significant.



**Extended Data Fig. 4: A-** Net MHCpan 4,1 shows that the antigen FLIIWQNTM resulting from protein caspase-5 can bind to HLA-A*02 complex albeit weakly (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1)

**B-** Net Chop 3.1 shows the FLIIWQNTM peptide has potential cleavage sites in its sequence which may result in different peptide sequence being presented by the MHC-I complex (https://services.healthtech.dtu.dk/service.php?NetChop-3.1). The predicted cleavage sites are denoted by arrows. These are after the assigned 'S' i.e. the peptide-bond on the C-terminal side of an amino acid with an assigned 'S' is cleaved.

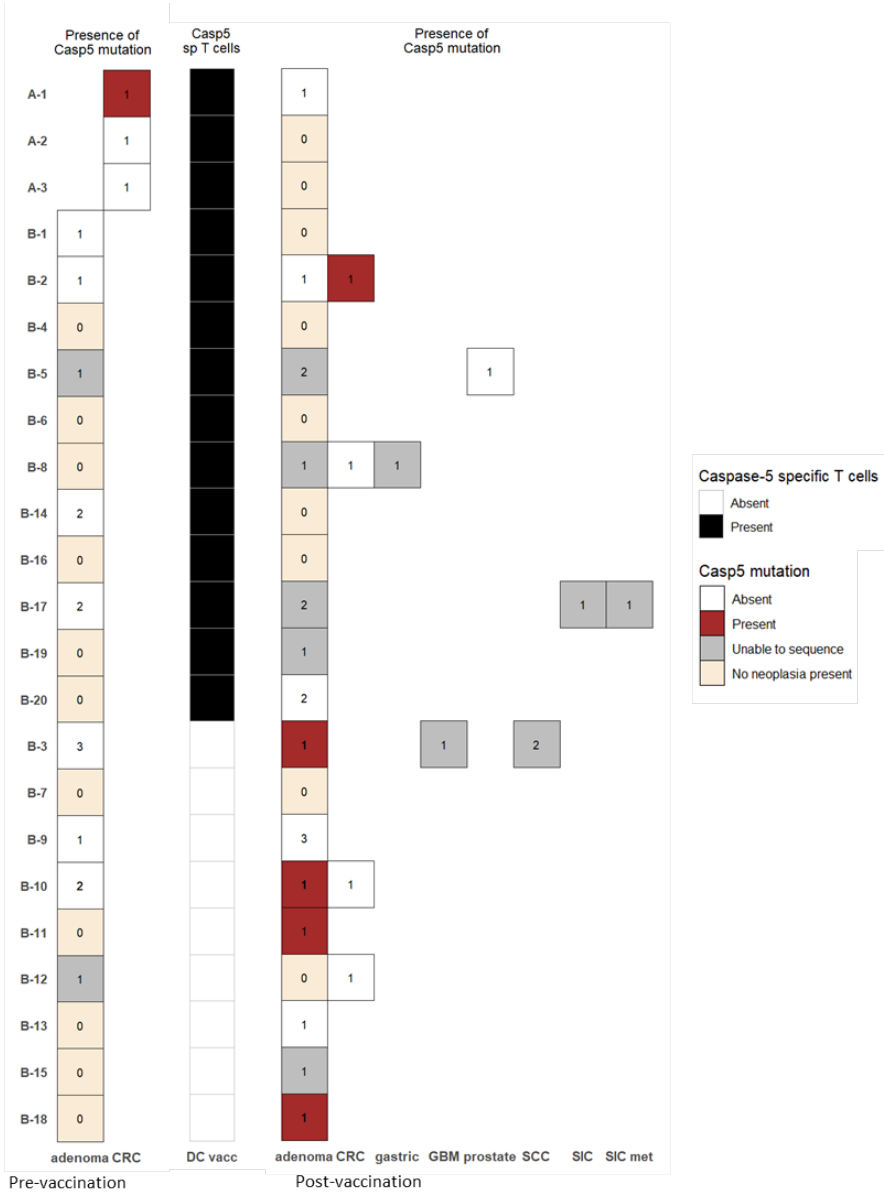
A

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 70 | HLA-A*02:01 | RCWNTWAKM | RCWNTWAKM | 0 | 0 | 0 | 0 | 0 | RCWNTWAKM | Sequence 0.0030980 | 11.321 |
| 71 | HLA-A*02:01 | CWNTWAKMF | CWNTWAKMF | 0 | 0 | 0 | 0 | 0 | CWNTWAKMF | Sequence 0.0000130 | 70.000 |
| 72 | HLA-A*02:01 | WNTWAKMFF | WNTWAKMFF | 0 | 0 | 0 | 0 | 0 | WNTWAKMFF | Sequence 0.0000160 | 67.500 |
| 73 | HLA-A*02:01 | NTWAKMFFM | NTWAKMFFM | 0 | 0 | 0 | 0 | 0 | NTWAKMFFM | Sequence 0.0294380 | 3.923 |
| 74 | HLA-A*02:01 | TWAKMFFMV | TWAKMFFMV | 0 | 0 | 0 | 0 | 0 | TWAKMFFMV | Sequence 0.0021200 | 13.265 |
| 75 | HLA-A*02:01 | WAKMFFMVF | WAKMFFMVF | 0 | 0 | 0 | 0 | 0 | WAKMFFMVF | Sequence 0.0000140 | 69.167 |
| 76 | HLA-A*02:01 | AKMFFMVFL | AKMFFMVFL | 0 | 0 | 0 | 0 | 0 | AKMFFMVFL | Sequence 0.0012430 | 16.430 |
| 77 | HLA-A*02:01 | KMFFMVFLI | KMFFMVFLI | 0 | 0 | 0 | 0 | 0 | KMFFMVFLI | Sequence 0.2258100 | 1.022 <= WB |
| 78 | HLA-A*02:01 | MFFMVFLII | MFFMVFLII | 0 | 0 | 0 | 0 | 0 | MFFMVFLII | Sequence 0.0003810 | 25.854 |
| 79 | HLA-A*02:01 | FFMVFLIIW | FFMVFLIIW | 0 | 0 | 0 | 0 | 0 | FFMVFLIIW | Sequence 0.0000430 | 53.333 |
| 80 | HLA-A*02:01 | FMVFLIIWQ | FMVFLIIWQ | 0 | 0 | 0 | 0 | 0 | FMVFLIIWQ | Sequence 0.0001480 | 36.250 |
| 81 | HLA-A*02:01 | MVFLIIWQN | MVFLIIWQN | 0 | 0 | 0 | 0 | 0 | MVFLIIWQN | Sequence 0.0001120 | 39.778 |
| 82 | HLA-A*02:01 | VFLIIWQNT | VFLIIWQNT | 0 | 0 | 0 | 0 | 0 | VFLIIWQNT | Sequence 0.0000550 | 49.500 |
| 83 | HLA-A*02:01 | FLIIWQNTM | FLIIWQNTM | 0 | 0 | 0 | 0 | 0 | FLIIWQNTM | Sequence 0.3433800 | 0.646 <= WB |

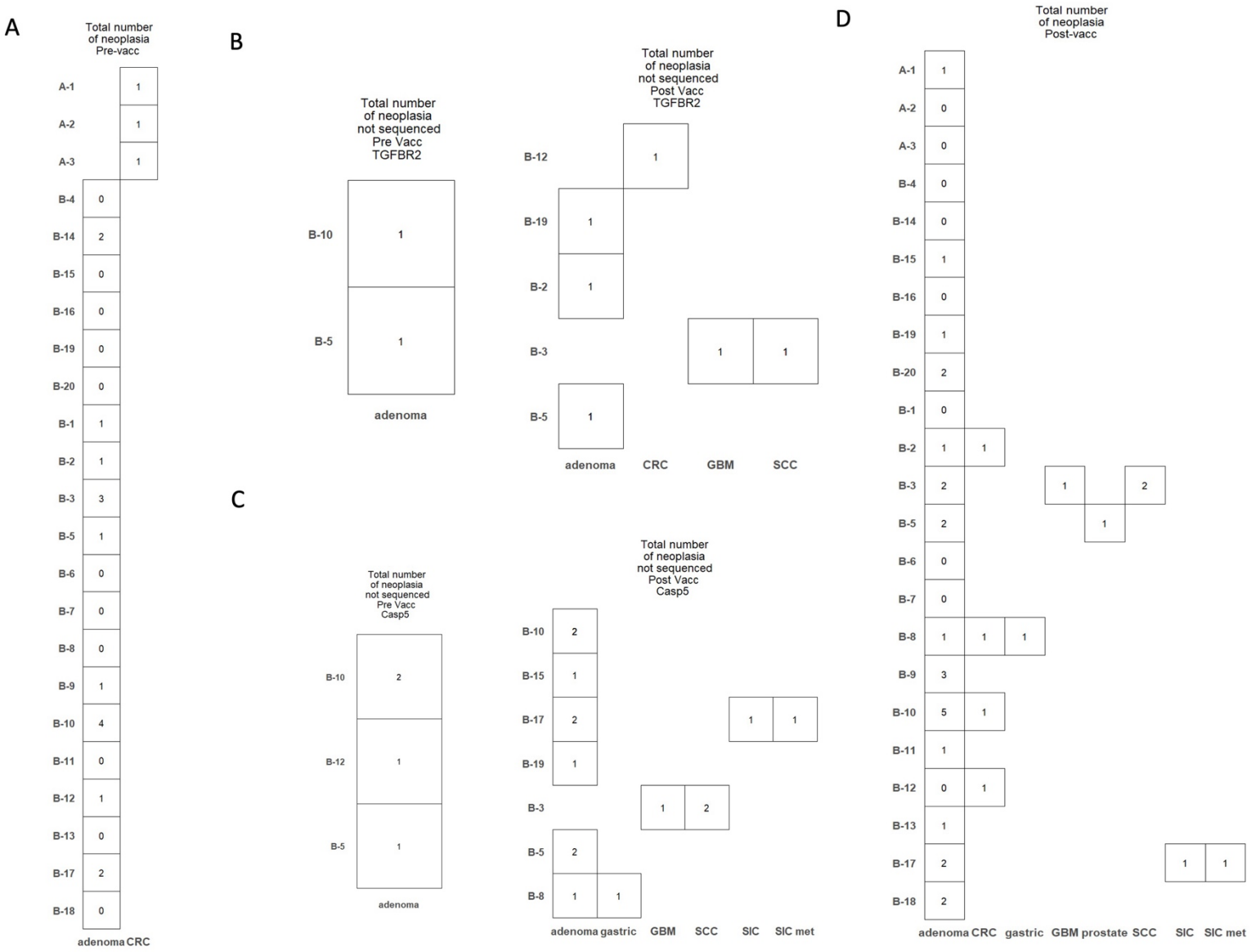
B

| | | | | |
|---|---|---|---|---|
| 66 | K | S | 0.892591 | Sequence |
| 67 | K | S | 0.857451 | Sequence |
| 68 | Q | . | 0.071516 | Sequence |
| 69 | L | . | 0.460360 | Sequence |
| 70 | R | . | 0.060011 | Sequence |
| 71 | C | . | 0.023769 | Sequence |
| 72 | W | . | 0.206570 | Sequence |
| 73 | N | . | 0.022555 | Sequence |
| 74 | T | . | 0.026255 | Sequence |
| 75 | W | . | 0.216398 | Sequence |
| 76 | A | . | 0.048999 | Sequence |
| 77 | K | S | 0.549278 | Sequence |
| 78 | M | . | 0.349941 | Sequence |
| 79 | F | . | 0.052692 | Sequence |
| 80 | F | . | 0.065524 | Sequence |
| 81 | M | . | 0.097618 | Sequence |
| 82 | V | . | 0.083763 | Sequence |
| 83 | F | . | 0.165187 | Sequence |
| 84 | L | . | 0.439660 | Sequence |
| 85 | I | . | 0.149879 | Sequence |
| 86 | I | . | 0.288824 | Sequence |
| 87 | W | S | 0.771986 | Sequence |
| 88 | Q | . | 0.048356 | Sequence |
| 89 | N | . | 0.291842 | Sequence |
| 90 | T | . | 0.164071 | Sequence |
| 91 | M | S | 0.948030 | Sequence |

**Extended Data Fig. 5:** Overview of the mutation in *CASP5* gene seen for adenoma or cancer samples sequenced for all patients before and after DC vaccination. The number inside the boxes refers to the total number of sequenced tumors (obtained at different time points from the patients). If mutation in *CASP5* gene has been detected in at least one tumor, the block is colored red. The presence or absence of caspase-5 specific CD8 T-cells as seen in the DTH test is plotted to make correlation with immune response observed in these patients. No correlation was seen with caspase-5 specific CD8 T-cells and the development of adenomas or cancers post-vaccination. CRC: Colorectal cancer; GBM: Glioblastoma; SCC: squamous cell carcinoma; SIC: Small intestinal cancer; SIC met: metastasis of small intestinal cancer



**<u>Extended Data Fig. 6:</u>** Overview of the tissues obtained from the patients in the trial **A-** before the vaccination and **D-** after vaccination. Some tissues could not be sequenced for detecting the mutation in **B-** *TGFBR2* gene and **C-** *CASP5* gene. The tissues could not be sequenced due to their extremely small size and low quality of DNA obtained from them. CRC: Colorectal cancer; GBM: Glioblastoma; SCC: squamous cell carcinoma; SIC: Small intestinal cancer; SIC met: metastasis of small intestinal cancer



Extended Data Table 1: Patient baseline characteristics

| | **Lynch Syndrome associated colorectal cancer patients**<br><br>**n=3** | **Lynch Syndrome mutation carriers n=20** |
|---|---|---|
| **Sex** | | |
| **Male** | **2 (67%)** | **13 (65%)** |
| **Female** | **1 (33%)** | **7 (35%)** |
| **Age (years)** | | |
| **Mean (range)** | **47 (46-48)** | **51 (29-65)** |
| **Germline mutation** | | |
| ***MLH1*** | **1 (33%)** | **9 (45%)** |
| ***MSH2*** | **1 (33%)** | **7 (35%)** |
| ***MSH6*** | **1 (33%)** | **3 (15%)** |
| ***PMS2*** | | **1 (5%)** |
| **Previous Cancer** | | |
| **LS-associated cancer** | **3 (100%)[#]** | **2 (10%)[+]** |
| **Tumor stage** | **A-1 CRC pT3N0M0** | **B-3 UTUC pTaG2a** |
| | **A-2 CRC pT2N0M0** | **B-10 CRC Dukes C2 T3N1-2M0** |

| | | |
|---|---|---|
| | **A-3 CRC pT1N0M0** | |
| **Cancer-related treatment** | | |
| **Surgical resection** | **3 (100%)** | **3 (15%)** |
| **Adjuvant chemotherapy** | **-** | **1 (5%)** |
| **Other adjuvant treatment** | **-** | **-** |

CRC: colorectal cancer; UTUC: upper tract urothelial cancer

# CRC treated surgically within one year prior to vaccination

+ All cancers were diagnosed at least five years before study enrollment

Extended Data Table 2: Adverse events post-vaccination

| **Lynch Syndrome patients (n=23)** | **Number of patients (percent)** | | | |
|---|---|---|---|---|
| | **All grades** | **Grade 1-2** | **Grade 3** | **Grade 4** |
| **Vaccine-related toxicity^** | | | | |
| **Flu-like symptoms#** | **23 (100%)** | **23 (100%)** | **0 (0%)** | **0 (0%)** |
| **Injection site reaction** | **19 (83%)** | **19 (83%)** | **0 (0%)** | **0 (0%)** |
| **Fever** | **12 (52%)** | **11 (48%)** | **0 (0%)** | **1 (4%)** |
| **Anemia** | **9 (39%)** | **9 (39%)** | **0 (0%)** | **0 (0%)** |
| **Hypoalbuminemia** | **8 (35%)** | **8 (35%)** | **0 (0%)** | **0 (0%)** |
| **Elevation in AST level** | **7 (30%)** | **7 (30%)** | **0 (0%)** | **0 (0%)** |
| **Hypokalemia** | **6 (26%)** | **6 (26%)** | **0 (0%)** | **0 (0%)** |
| **Elevation in ALT level** | **6 (26%)** | **6 (26%)** | **0 (0%)** | **0 (0%)** |
| **Hypophosphatemia** | **4 (17%)** | **3 (13%)** | **1 (4%)** | **0 (0%)** |
| **Elevation in GGT level** | **7 (30%)** | **7 (30%)** | **0 (0%)** | **0 (0%)** |
| **Hyperbilirubinemia** | **4 (17%)** | **4 (17%)** | **0 (0%)** | **0 (0%)** |
| **Inflamed DTH-biopsy place** | **3 (13%)** | **3 (13%)** | **0 (0%)** | **0 (0%)** |
| **Pneumonia** | **2 (9%)** | **2 (9%)** | **0 (0%)** | **0 (0%)** |
| **Vomiting** | **2 (9%)** | **2 (9%)** | **0 (0%)** | **0 (0%)** |

| | | | | |
|---|---|---|---|---|
| **Elevation in ALP level** | **2 (9%)** | **2 (9%)** | **0 (0%)** | **0 (0%)** |
| **Hypomagnesemia** | **2 (9%)** | **2 (9%)** | **0 (0%)** | **0 (0%)** |
| **Hypocalcemia** | **2 (9%)** | **2 (9%)** | **0 (0%)** | **0 (0%)** |
| **Nausea** | **1 (4%)** | **1 (4. %)** | **0 (0%)** | **0 (0%)** |
| **Fatigue <** | **1 (4 %)** | **1 (4%)** | **0 (0%)** | **0 (0%)** |
| **Hyponatremia** | **1 (4%)** | **1 (4%)** | **0 (0%)** | **0 (0%)** |

ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DTH: delayed-type hypersensitivity; GGT: gamma-glutamyl transferase

^ attributed by investigators.

# flu-like symptoms include fever, fatigue, chills, body aches, malaise, loss of appetite and headache.

< fatigue was mentioned separately when it lasted at least 1 day longer than the other flu-like symptoms or when it was present without the other flu-like symptoms.

Extended Data Table 3: Clinical Outcome

| DC treated patient | Cancer during follow up | Cancer related treatment | Disease free survival (months) | Overall survival (months) |
|---|---|---|---|---|
| A-1 | - | - | 138.9 | 138.9 |
| A-2 | - | - | 112.0 | 112.0 |
| A-3 | - | - | 103.6 | 103.6 |
| B-1 | - | - | 82.6 | 82.6* |
| B-2 | CRC# | S | 68.1* | 111.8 |
| B-3 | SCC, GBM | S, pRT | 33.3* | 76.9* |
| B-4 | - | - | 112.2 | 112.2 |
| B-5 | PCa# | S, sRT | 24.5* | 108.4 |
| B-6 | - | - | 91.1 | 91.1 |
| B-7 | - | - | 105.0 | 105.0 |
| B-8 | CRC#, GC# | S, pCT | 2.3* | 12.2* |
| B-9 | - | - | 98.7 | 98.7 |
| B-10 | CRC# | S | 66.7* | 108.8 |
| B-11 | - | - | 97.8 | 97.8 |
| B-12 | CRC# | S | 5.3* | 108.4 |
| B-13 | - | - | 107.3 | 107.3 |

| | | | | |
|---|---|---|---|---|
| **B-14** | **-** | **-** | **102.6** | **102.6** |
| **B-15** | **-** | **-** | **102.8** | **102.8** |
| **B-16** | **-** | **-** | **107.4** | **107.4** |
| **B-17** | **SIC** | **S** | **63.2*** | **70.7** |
| **B-18** | **-** | **-** | **101.3** | **101.3** |
| **B-19** | **-** | **-** | **102.9** | **102.9** |
| **B-20** | **-** | **-** | **96.3** | **96.3** |

CRC: Colorectal cancer; GBM: Glioblastoma; PCa: prostate cancer; SCC: squamous cell carcinoma; GC: Gastric Cancer; SIC: Small Intestinal Cancer; S: surgery; sRT: salvage radiotherapy; pCT: palliative chemotherapy; pRT: palliative radiotherapy

**The last follow-up date was January 1, 2024. Patients who had not reached a DFS or OS endpoint by this date were censored at last follow-up**

# Lynch syndrome-associated cancer confirmed by mismatch repair protein deficiency immunohistochemistry

* DFS or OS end point reached in this patient