Supplemental Data

Bhatia et al. Intratumoral delivery of plasmid interleukin-12 via electroporation leads to regression of injected and non-injected tumors in Merkel cell carcinoma

**Screening &**

**Enrollment**

**Day 1**

**Day 5**

**Day 8**

**Day 22**

**Pre-treatment**

**Tumor Biopsy and Blood**

**(Day 1)**

**Post-treatment**

**Tumor Biopsy**

**(Surgery in Cohort A)**

**and Blood**



**Cohort A**

**Cohort B**

**Definitive Surgery in week 4**

**(+/- adjuvant Radiation)**

**Long-term follow up**

**Long-term follow up**

**i.t.-tavo-EP treatment**

**Additional Treatment**

**Cycles (up to 4 total)**

**every 6 weeks**

**Supplemental Figure 1. Treatment schema for i.t.-tavo-EP.** Plasmid IL-12 (Syringe) was injected intra-tumorally followed immediately by electroporation (Bolt) on Days 1, 5, and 8. Patients with locoregional MCC (**Cohort A**) received one cycle before definitive surgery during week four with or without adjuvant radiation therapy and then followed long-term. Patients with distant metastatic disease (**Cohort B**) could receive up to four cycles total, administered at least six weeks apart and then followed long-term. Serial tumor biopsies and peripheral blood samples were collected from all patients to evaluate cellular and humoral immunologic changes, including changes in IL-12 protein expression in the tumor microenvironment.

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| **Supplemental Table 1: Tetramer staining summary** | | | | | | | | | | | | | |
| **Patient**  **ID** | **Clinical Response** | **Viral status** | **A-1** | **A-2** | **B-1** | **B-2** | **Tet Response** | **Pre-Tx PBMC (% Tet+)** | **Post-Tx PBMC**  **(% Tet+)** | **Fold change, PBMC** | **Pre-Tx TIL**  **(% Tet+)** | **Post-Tx TIL**  **(% Tet+)** | **Fold change, TIL** |
| 1 | PD | - | A03 | A68 | B14 | B51 | N/A | No tetramer | No tetramer | N/A | No tetramer | No tetramer | N/A |
| 2 | PR | + | A03 | A30 | B13 | B35 | Neg | Negative | Negative | Neg | 0.014 | 0.1 | 6.14 |
| 3 | PD | + | A01 | A02 | B35 | B63 | A02 | 0.070 | 0.120 | 0.71 | No sample | No sample | N/A |
| B35 | 0.012 | 0.025 | 1.08 | No sample | No sample | N/A |
| 4 | PD | + | A02 | A24 | B14 | B44 | A02 | 0.038 | 0.049 | 0.29 | No sample | A02 neg | N/A |
| A24 | 0.300 | 0.800 | 1.67 | No sample | A24(0.11) | N/A |
| 5 | PD | - | A02 | A03 | B07 | B44 | Neg | Negative | Negative | Neg | Negative | Negative | Neg |
| 6 | SD | + | A02 | A26 | B40 | B49 | A02 | 0.120 | 0.130 | 0.08 | No sample | A02(1.43) | N/A |
| 7 | PD | + | A02 | A24 | B15 | B40 | A02 | 0.230 | 0.082 | -0.64 | 0.019 | 0.014 | -0.26 |
| A24 | 0370 | 0.510 | 0.38 | 1.680 | 0.510 | -0.70 |
| 8 | PR | + | A01 | A24 | B07 | B35 | A24 | No Pre-tx PBMC | Negative | N/A | 0.034 | 0.055 | 0.62 |
| B07 | No Pre-tx PBMC | Negative | N/A | 0.027 | 0.044 | 0.63 |
| B35 | No Pre-tx PBMC | Negative | N/A | 0.029 | 0.390 | 12.45 |
| 10 | PD | + | A02 |  | B35 | B44 | A02 | Negative | Negative | Neg | Negative | Negative | Neg |
| B35 | Negative | Negative | Neg | Negative | Negative | Neg |
| 11 | PD | + | A02 |  | B51 |  | Neg | Negative | Negative | Neg | Negative | No Bx for TIL | N/A |
| 12 | SD | + | A01 | A30 | B08 | B18 | N/A | No tetramer | No tetramer | N/A | No tetramer | No tetramer | N/A |
| 13 | PR | + | A03 | A24 | B07 | B51 | A24 | 0.130 | 1.580 | 11.15 | 0.290 | 1.110 | 2.83 |
| B07 | Negative | Negative | Neg | 0.052 | 0.068 | 0.31 |
| 14 | PR | - | A03 | A24 | B14 | B35 | Neg | Negative | Negative | Neg | Negative | Negative | Neg |
| 15 | PD | + | A03 | A24 | B35 | B40 | Neg | Negative | Negative | Neg | Negative | No Bx for TIL | N/A |
| 16a | CR | + | A02 | A03 | B37 | B44 | A02 | 0.096 | 0.043 | -0.55 | 3.410 | 1.360 | -0.60 |
| B37 | 0.610 | 0.480 | -0.21 | 20.000 | 8.930 | -0.55 |
| A02 |  |  |  | 3.410 | 4.700 | 0.38 |
| B37 |  |  |  | 20.000 | 29.700 | 0.49 |
| a Pre-treatment biopsy did not contain tumor. Excluded from TIL analyses.  Clinical response listed as either complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD).  HLA alleles for both A and B subtypes are listed (A-1, A-2, B-1, B-2).  The HLA allele type tested is listed under tet response. If no tetramer population was detectable above 0.001% frequency of CD8 T cells, they were listed as negative. If a patient did not have an HLA type with a corresponding MCPyV tetramer, they were listed as ‘N/A’. The frequencies of MCPyV tetramer positive CD8 T cells (% Tet+) for both PBMC and TIL are reported as a percentage of all CD8 cells. | | | | | | | | | | | | | |

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| **Supplemental Table 2: Characteristics and outcomes in patients who received PD-1 blockade at some point after receiving i.t.-tavo-EP** | | | | | |
| **Patient #**  **(Cohort)** | **Age (Years);**  **Gender;**  **MCPyV Sero-Statusa** | **Best response to i.t.-tavo-EP;**  **Duration of clinical benefit (months)** | **Time to PD-1 blockade (months);**  **Drug(s) given** | **Post-i.t.-tavo-EP therapies**  **(in order of administration)** | **Best response to PD-1 blockade; Duration of benefit (months)** |
| 2  (B) | 56; M;  Positive | PR;  18 | 15;  MCPyV-specific T-cells + pembrolizumab + Ipilimumab | RT; SSA ; 4-1bb agonist; cytotoxic chemotherapy | PR (>90% regression);  21 |
| 8  (A) | 60; M;  Positive | NE;  9 | 30;  pembrolizumab | Surgery; RT; IT IFN-b | PR;  25 |
| 12  (B) | 67; M;  Positive | SD;  3 | 19;  nivolumab | IT TLR-4 agonist; 4-1bb agonist; cytotoxic chemotherapy; RT; pazopanib | CR;  26 |
|
| 14  (B) | 63; M;  Negative | PR;  3 | 5;  pembrolizumab | SRS to brain metastasis | CR;  44 |
|
| 15  (B) | 73; F;  Negative | PD;  2 | 2;  pembrolizumab | None | CR;  48+ (ongoing) |
| a As determined by presence of serum antibodies to viral T-protein (not capsid proteins).  Abbreviations: CR – Complete response; F – Female; IFN-b: Interferon-beta; IT – Intratumoral; i.t.-tavo-EP – intratumoral injection of interleukin-12 plasmid DNA (tavo) followed by *in vivo* electroporation; M – Male; NE – Not evaluable; RT – Radiation therapy; PD – Progressive disease; PD-1 – Programmed death-1; PR – Partial response; SD – Stable disease; SSA – Somatostatin analogues; TLR – Toll-like receptor; SRS – stereotactic surgery | | | | | |