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  |