

SUPPLEMENTARY INFORMATION

Generation of Immunity to the Folate Receptor Alpha in Breast and Ovarian Cancer Patients Following Active Immunization with a Folate Receptor Alpha Peptide Vaccine

Kimberly R. Kalli^{1*}, Matthew S. Block^{1,2*}, Pashtoon M. Kasi¹, Courtney L. Erskine², Timothy J. Hobday¹, Allan B. Dietz³, Douglas J. Padley³, Michael P. Gustafson³, Dan W. Visscher³, Danell J. Puglisi-Knutson², Barath Shreeder², Toni K. Mangskau⁴, Glynn Wilson⁵, and Keith L. Knutson².

¹Department of Oncology, ²Department of Immunology, ³Department of Laboratory Medicine and Pathology, and the ⁴Mayo Clinic Cancer Education Program, Mayo Clinic, Rochester, MN 55905 and Jacksonville FL 32224. ⁵Tapimmune, Inc., Jacksonville, FL 32202

Correspondence: Dr. Keith L. Knutson, Professor of Immunology, Department of Immunology, Mayo Clinic, 4500 San Pablo Rd. Jacksonville, FL 32224, 904-953-6657, knutson.keith@mayo.edu

SUPPLEMENTARY RESULTS

Vaccination generates T cell but not antibody immunity to FR α following immunization

The frequency of IFN- γ -producing T cells specific for FR α peptides, FR α protein, TT, and control peptide were compared between pre-treatment samples and multiple post-treatment samples, both during vaccine treatment (up to 30 days following the last vaccination) using the FBS-based ELIspot. T cell immunity was assessed in 15 of 21 patients. In Supplementary Fig. 1A the pre-immunization and highest post-immunization T cell frequencies are plotted, excluding values from the observation period at 3, 6, and 12 months following the final vaccination. The median number of post-vaccination (not including observations) period samples was 6 (range 2-6). The pre-immunization IFN- γ T cell frequency to FR30 was 229 ± 65 (\pm s.e.m., n=14) T cells/million PBMCs which increased to 419 ± 75 T cells/million PBMCs ($p=0.002$). To FR56, T cells increased from 210 ± 67 to 407 ± 88 ($p=0.031$), to FR76 from 68 ± 29 to 300 ± 63 ($p=0.006$), to FR113 from 40 ± 12 to 299 ± 63 ($p=0.04$), to FR238 from 99 ± 47 to 249 ± 61 ($p=0.05$). There was a minor and insignificant increase in the reactivity to the pan-DR binding cyclin D1 peptide from 45 ± 20 to 76 ± 15 ($p=0.2$). For FR α , the mean frequency increased 142 ± 46 to 333 ± 78 T cells ($p=0.019$), which indicates that the vaccine is generating T cells that are recognizing naturally processed antigens. Lastly, the mean TT T cell frequency increased from 590 ± 68 to 728 ± 95 TT-specific T cells per million PBMC, although this increase was not significant likely due to the low SNR ($p=0.2$) (Supplementary Fig. 1B).

Supplementary Fig. 1: Vaccination generates T cell immunity to FR α following immunization. **Panel A** shows the mean (n=15 patients) pre-immunization (Pre) and highest post-vaccination (Post) frequency of antigen-specific T cells frequencies (per million PBMC plated) that recognize vaccine antigens, FR30, FR56, FR76, FR113, and FR238. Assay was done using the FBS-based ELIspot. Also shown are frequencies to control cyclin D1 and the FR α protein. **Panel B** shows the mean pre-immunization (Pre) and highest post-vaccination (Post) frequency of tetanus toxoid-specific T cells for same patients in Panel A. p values shown were calculated using the paired student's T test. Each line traces the pre- and post-antigen-specific T cell levels for a single unique patient measured during the vaccine period.

Supplementary Fig. 1

