Supporting Information

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SI Materials and Methods

Vaccine Production and Treatment. Tumor biopsy specimens were prepared as sterile single cell suspensions containing 10% DMSO and stored in liquid nitrogen. RNA was isolated from approximately 5×10^6 cells using standard methods (RNeasy; Qiagen). Poly(A)-positive mRNA was isolated with oligo(dT) beads (Dynal) and cDNA was produced by standard techniques. The tumor-specific variable regions were amplified by polymerase chain reaction (PCR) using 5′ RACE (rapid amplification of cDNA ends) of G-tailed cDNA with C anchor 5′ primer and C_H1 specific 3′ primers (1, 2). Amplifications were performed in duplicate. Tumor gene sequences were obtained by direct sequencing of the PCR product and verified by cloning and subsequent sequencing of 48 of the independent isolates of each cloned chain. Cloned patient variable region genes and specific primers were used to assemble the scFv by PCR where the linker region of the scFv was generated by amplification using primers containing a semirandom sequence (3).

PCR-generated inserts were ligated into a modified tobacco mosaic virus (TMV) expression vector (4–6) to create a set of ~100 idiotype-derived scFv, each containing a variable linker sequence. These scFv constructs were subsequently screened *in planta* for expression and folding optimization as described previously (3). For each patient, the TMV expression vector containing the optimized scFv expression construct that yielded high levels of full-length protein was transcribed *in vitro* using a T7 promoter in the vector. The resulting RNA transcripts were inoculated onto *Nicotiana benthamiana* plants using a mild abrasive to facilitate virus infection. Eleven days after inoculation, leaves from 1,000 plants were harvested and subjected to vacuum infiltration as previously described (7). Interstitial fluid (IF) containing the secreted scFv protein was recovered by centrifugation, filtered, concentrated, and subjected to ion exchange chromatography. Fractions containing the scFv were pooled, brought to 25% ammonium sulfate and purified on Phenyl Sepharose HP (Amersham Pharmacia) followed by Hydroxyapatite Type I chromatography (Bio-Rad). Fractions containing purified scFv product were pooled, concentrated and diafiltered into phosphate-buffered saline (PBS), pH 7.4 and sterile filled into polypropylene vials. In one case, Patient #16, a His-tag version of the scFv protein was produced and purified using nickel affinity chromatography as the initial capture step. All scFv vaccines were manufactured under FDA current good manufacturing practice (cGMP) guidelines.

Vaccine Release and Glycan Analysis. The quality of vaccine products was monitored using cGMP Quality Control (QC) and Quality Assurance (QA) guidelines. Product release assays were used to assess product identity and tumor relevance, purity, potency, and other product properties including safety. The absence of infectious TMV vector was confirmed by local lesion assay using *Nicotiana tabacum* NN leaf inoculation methods. The quality of the vaccine products was also monitored during a 12–24 month stability study using a subset of the product release tests used for clinical application (8).

Glycan analysis, presented in Table 2, was carried out as described in detail elsewhere (9, 10). Briefly, each patient's scFv sequence was analyzed for expected glycosylation sites based on the presence of an N-glycosylation sequence, Asn-X-Ser/Thr. Patient scFv protein was analyzed by MALDI-TOF mass analysis, and initially the number of glycosylation sites occupied were determined by the difference in mass from the expected amino acid weight based on protein sequence and observed mass, divided by the average glycan mass of 1,220 Da. Glycosylation is also clearly observed in the differential migration of scFv proteins by SDS-PAGE analysis (Fig. 1). Additional analyses by tryptic digestion of scFv protein, to generate peptide fragments predicted to contain N-liked glycosylation sites, were further analyzed by MALDI-TOF. The presence of glycan occupation was confirmed, and the mass of each glycan was established (9). The exact composition of glycan identity will be presented elsewhere (F.V., K.M.H., and E.L.W., unpublished data).

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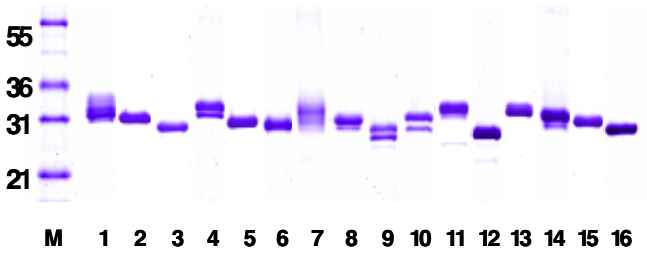

Fig. S1. SDS-PAGE analysis of independently produced scFv vaccines. Each scFv vaccine from final formulation vial was separated by SDS-PAGE (1 μg/lane; Invitrogen 10–20% Tris-Glycine gel) and stained with Coomassie Brilliant Blue. A molecular weight marker (Mark 12) is shown in lane marked ''M'' and vaccines are numbered corresponding to the patient number (1–16) that received the materials.

Table S1. Vaccine release specifications and associated testing methods

Test	Specification
Release	
SDS-PAGE/densitometry	Proteins were separated by SDS-PAGE, stained with Coomassie Brilliant Blue, and measured using calibrated imaging densitometry. 90% protein in the molecular weight range of 28.5 kDa, ± 20% and of truncated species as calculated (including truncated species, if present). If truncated species are used to obtain 90% protein specification, they must be verified by tryptic peptide mass mapping.
N-terminal amino acid sequence	The first 10 residues of sequence must match the predicted N-terminal sequence with a maximum of one unresolved residue.
Molecular weight	The MALDI-TOF MS molecular mass measurement must match either the calculated molecular mass or the calculated mass + allowance for glycosylation (~1200 Da, each oligosaccharide) based on predicted number of glycosylation sites.
Tryptic peptide mass measurement	The molecular masses for at least three different unmodified tryptic peptide fragments, determined by MALDI-TOF MS, must match the corresponding theoretical unmodified tryptic peptide fragments.
Appearance	Clear, colorless solution, free of particulates.
Endotoxin	Endotoxin < 10 EU/dose
Residual DNA	<10 ng/dose
Sterility	Sterile as judged by 21 CFR610.12 assay.
pH	7.3 ± 0.3 pH units
Protein concentration	Final vial concentration must be: 4.0 mg/ml ± 0.8 mg/ml OR 0.4 mg/ml ± 0.08 mg/ml, as indicated.
For information only	
Small-molecule impurities	Impurity concentration reported for 1-methyl-2-[3-pyridyl]-pyrrolidine (nicotine), phenyl methyl sulfonyl fluoride (PMSF) and 2-mercaptoethanol (BME).
Molecular weight and structural analysis of glycans	Glycan MW and structure reported by protease digestion of vaccines, enzymatic deglycosylation, glycan derivatization and analysis by LC-MS/MS using appropriate standards.
Infectious particles	Absence of infectious TMV reported by inoculation of *Nicotiana tabacum* NN plants with vaccines at a concentration of 4.0 mg/ml and lack of local lesions on inoculated leaves.