pNGVL4a-Sig/E7(detox)/HSP70

Plasmid DNA Expressing E7 for the Treatment of HPV-Associated Cervical Cancer

SUCCESS STORY

NSC 723254..............RECEIVED FEBRUARY 1999..............IRB APPROVAL Q4 2003..............CLINICAL TRIAL JANUARY 2004

RAID Application Received from Dr. Drew Pardoll, Johns Hopkins University 02/1999

- Consistent association of human papilloma virus (HPV) infection with development of cervical cancer.
- Consistent expression of HPV gene product E6 and E7 can be only found in cervical cancer but not normal cells.
- E6 and E7 are functionally required to cervical cancer phenotype.
- Cervical cancer is an ideal model for antigen-specific vaccination therapies.
- Applicant demonstrated that both recombinant DNA and vaccinia constructs containing LAMP-1-targeted E7 generate significantly enhanced E7-specific helper and CTL responses relative to vaccine constructs that contain wild-type E7.
- Both DNA and vaccinia recombinant vaccines employing sig-E7-LAMP-1 generate dramatically increased antitumor activity relative to E7 alone.

RAID Project Approved and Initiated 08/1999

- DTP made cell bank of the LAMP-1 construct.
- Dr. Pardoll requested a change in the DNA plasmid based on new preclinical data in which the LAMP-1 portion was replaced with heat shock protein 70 (HSP70).
- New construct: pNGVL4a-sig/E7(detox)/HSP70.

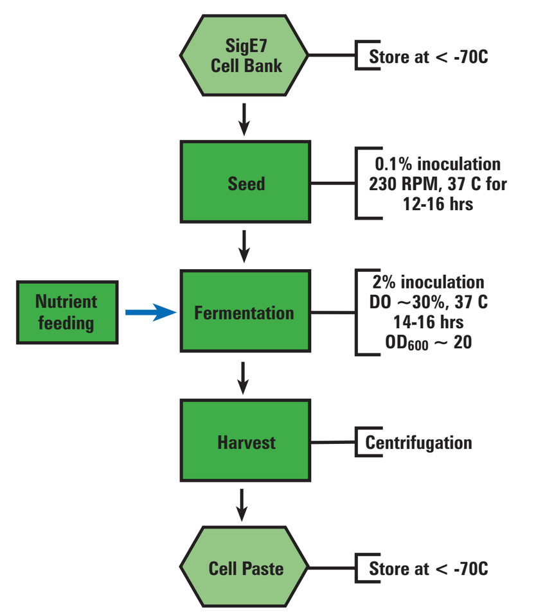
RAID Project Re-Reviewed Due to Substantial Change to Project 02/2001

- Dr. Pardoll presented data indicating that the E7/HSP70 construct produced strong E7-specific CD8 T-cell responses when administered either as a DNA, vaccinia, or DNA-DNA (prime-boost) regimen.
- This construct also produced strong antitumor immunity against E7-expressing TC-1 tumor either as a s.c. challenge or lung metastasis.
- Head-to-head comparisons of the LAMP-1 vs HSP70 indicated that the latter construct was more potent. The results of the re-review were for approval to proceed with the production of the pNGVL4a-sig/E7(detox)/HSP70.

Production Work Commenced Spring 2001

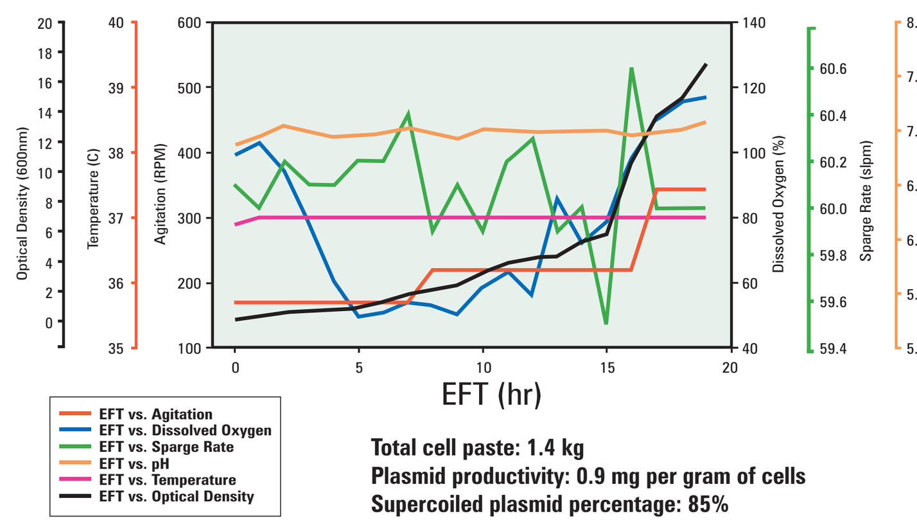
- DNA sequencing showed it contained an extraneous 36-amino acid tail.
- The plasmid for this construct was sent to the Biopharmaceutical Development Program (BDP).
- Based on review of the preclinical data generated at Johns Hopkins University and safety considerations (concern about the 36-amino acid tail), it was decided to proceed with the pNGVL4a-sig/E7(detox)/HSP70, which had a 7-amino acid tail similar to the pcDNA3 construct used by Dr. Wu in his published tumor protection experiments.

Fermentation Overview



pNGLV4a-Sig/E7(detox)/HSP70 Fermentation (80 L Scale Technology Transfer Run)

Tech Transfer 80 L #380005



Total cell paste: 1.4 kg
Plasmid productivity: 0.9 mg per gram of cells
Supercoiled plasmid percentage: 85%

pNGLV4a-Sig/E7(detox)/HSP70 Fermentation (80 L scale) Process Monitoring and Controlling

DO_2	30% on-line controlling by agitation 250-500 RPM
Sparge Rate	0.25-1.25 v/v/m
Dissolved Oxygen Control	Step 1: When DO drops to < 30%, increase sparge to 1.25 v/v/m Step 2: When DO drops to < 30%, increase agitation to 500 RPM
Temperature	37° C
Vessel Pressure	5 psig
pH	7 ± 0.1, on-line controlling with 30% NH_4OH
Foam	P2000 when necessary
Nutrient Feeding	When DO is > 30% and rising, and Glucose is < 1.5 g/L, feed at 1 L/hr until DO drops to < 30%. Total volume of feed medium is 20% of fermentor capacity
Harvest Criterion	The cells are harvested at stationary phase (when DO_2 is not decreasing and OD_{600nm} reading does not increase significantly)

GMP fermentations can be performed at a 20 or 1,000 liter scale.

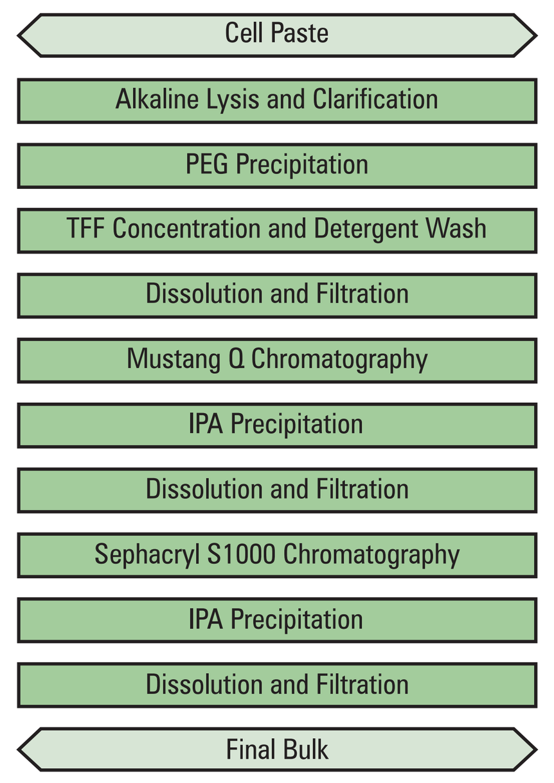
Preparations for final purification are performed under environmentally controlled conditions.

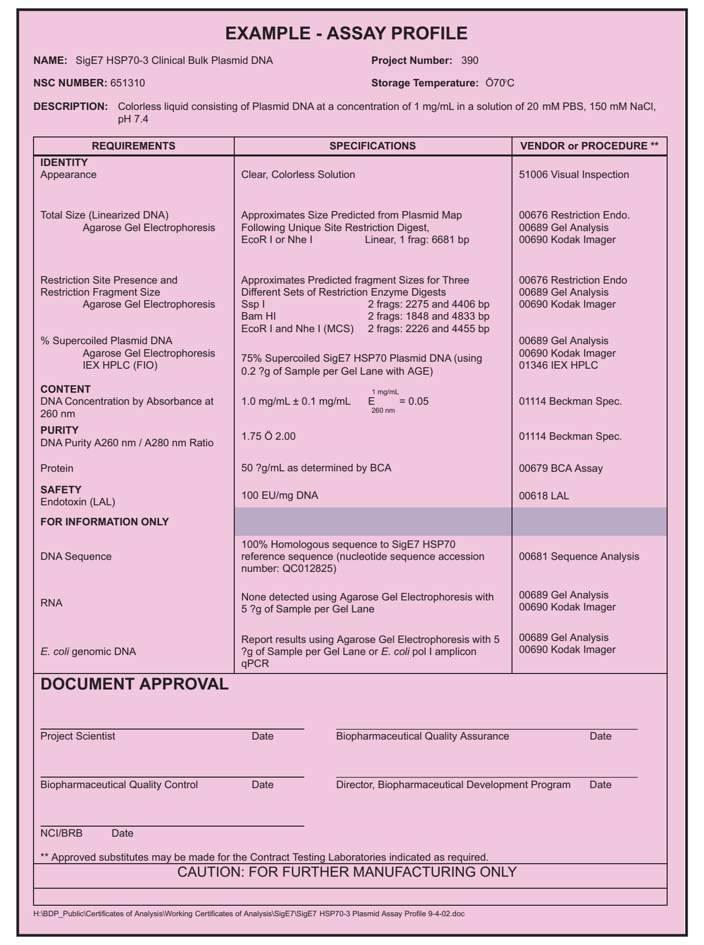
DNA Vaccine Ready for Testing in Cervical and Head & Neck Cancer Trials

NCI-supported researchers are beginning clinical trials of DNA-based cancer vaccines for cervical cancer and for head and neck cancer. The DNA vaccine effort was spearheaded by T.-C. Wu, M.D., Ph.D., and Drew Pardoll, Ph.D., researchers at Johns Hopkins Medical Institutions. They focused on human papillomavirus-16 (HPV-16), a strain of HPV responsible for more than 20 percent of head and neck cancers and more than half of all cervical cancers.

Purification Overview

pNGLV4a-Sig/E7(detox)/HSP70 Purification (1 kg scale) Process Overview


Cell Paste
Alkaline Lysis and Clarification
PEG Precipitation
TFF Concentration and Detergent Wash
Dissolution and Filtration
Mustang Q Chromatography
IPA Precipitation
Dissolution and Filtration
Sephacryl S1000 Chromatography
IPA Precipitation
Dissolution and Filtration
Final Bulk



pNGLV4a-Sig/E7(detox)/HSP70 Purification (1 kg scale) Process Description

Cell Lysis and Plasmid Extraction
Alkaline lysis is carried out using a static mixer. The lysate is clarified with depth filtration using 10" 5 μ filter unit. PEG 8000 is added to 8% to preferentially precipitate the plasmid DNA.

Tangential Flow Filtration
TFF (0.1 m) is used to concentrate and wash the DNA precipitate with detergent to remove endotoxin and other small molecule contaminants.

Mustang Q Chromatography
Dissolved plasmid DNA is captured on the Mustang Q capsule (membrane with quaternary amine groups). The capsule is washed with buffer containing detergent and eluted with salt.

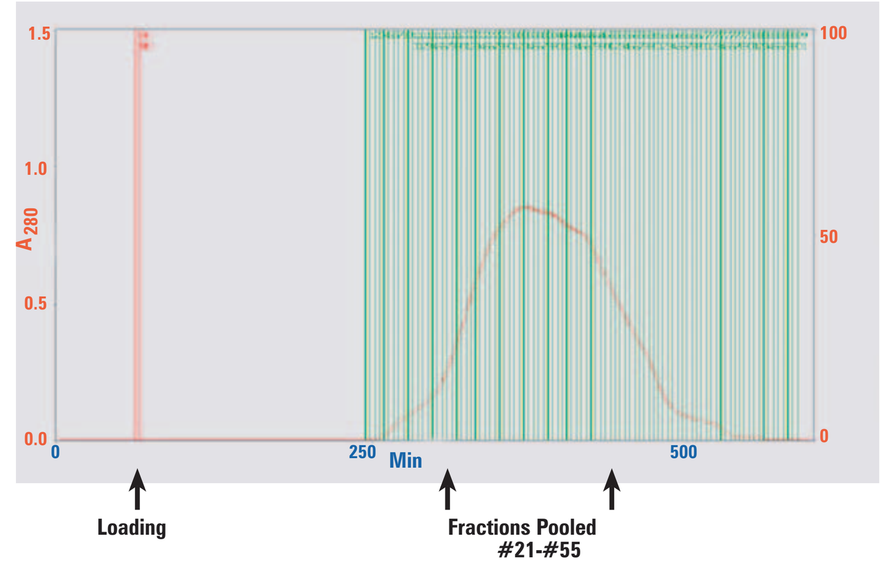
Sephacryl S1000 Chromatography
S1000 column is used as a final polishing step to reduce the level of genomic DNA and non-supercoiled plasmid.

pNGLV4a-Sig/E7(detox)/HSP70 Purification Development Runs Mustang Q Chromatography Lot 390016DEV (1 kg scale)

Step	Plasmid Recovered (mg)	S.C.
Alkaline lysis and purification PEG precipitation	910	86%
TFF concentration, wash, dissolution, and filtration	680	91%
Mustang Q chromatography	275	92%

Sephacryl S1000 Chromatography Lot 390008 (1.7 L column scale)

Step	Plasmid Recovered (mg)	S.C.
S1000 column load	103	92%
Fraction pool	78	>95%



Process Improvements

At least three subsequent RAID projects have benefitted from these process improvements.

		Qiagen Method	Methods Developed In-House		
			Project #390/#480	Project #447	Project #479
Process Overview		• Lysis • Qiagen column chromatography • Alcohol precipitation	• Batch lysis with RNase • PEG precipitation • Q-membrane chromatography • Size exclusion chromatography • Alcohol precipitation	• Continuous lysis • PEG precipitation • Q–convective flow chromatography • Hydrophobic/thlophiulic chromatography • TFF	• Continuous lysis • Q–convective flow chromatography • Hydrophobic interaction chromatography • TFF or alcohol precipitation
Product Quality		Acceptable (no % scDNA assurance)	High	High	High
Batch Scale	Cell Paste	< 10 g cell paste	1 kg cell paste	1 kg cell paste	2 kg cell paste
	Purified Plasmid	< 10 mg	100 – 500 mg	500 – 1000 mg	>1000 mg
Recovery		High	Low	Medium	High
Process Highlight and Improvement		Simple	• First in-house process successful in large-scale production	• Improved lysis procedure eliminated animal source material • More robust purification steps • Better recovery	• More robust purification steps • Better recovery • Reduced process time • Can be easily adapted to other projects

Toxicology Studies Initiated When Material Became Available 10/2002

- Mice were given 1, 10, or 100 μg/immunization each week for 3 weeks as an intramuscular dose. Mice were evaluated for the following parameters: clinical signs of toxicity, clinical pathology, and reversibility of macro- and microscopic lesions.
- No vaccine-related toxic effects were noted.

CMC and Toxicity Reports Supplied in April 2003

Dr. Pardoll Filed IND and Received IRB Approval Q4 2003

Clinical Trial Opened January 2004

Developmental Therapeutics Program of the National Cancer Institute

TURNING MOLECULES INTO medicines FOR THE public health