Recombinant DNA Advisory Committee for the Gene Transfer: Protocol #0210-556

Phase 1 Open-Label Dose Escalation Trial Evaluating the Safety and Immunogenicity of Sequential Administration of Recombinant DNA and Adenovirus Expressing L523S Protein in Patients with Early-Stage Non-Small Cell Lung Cancer

Sponsor:

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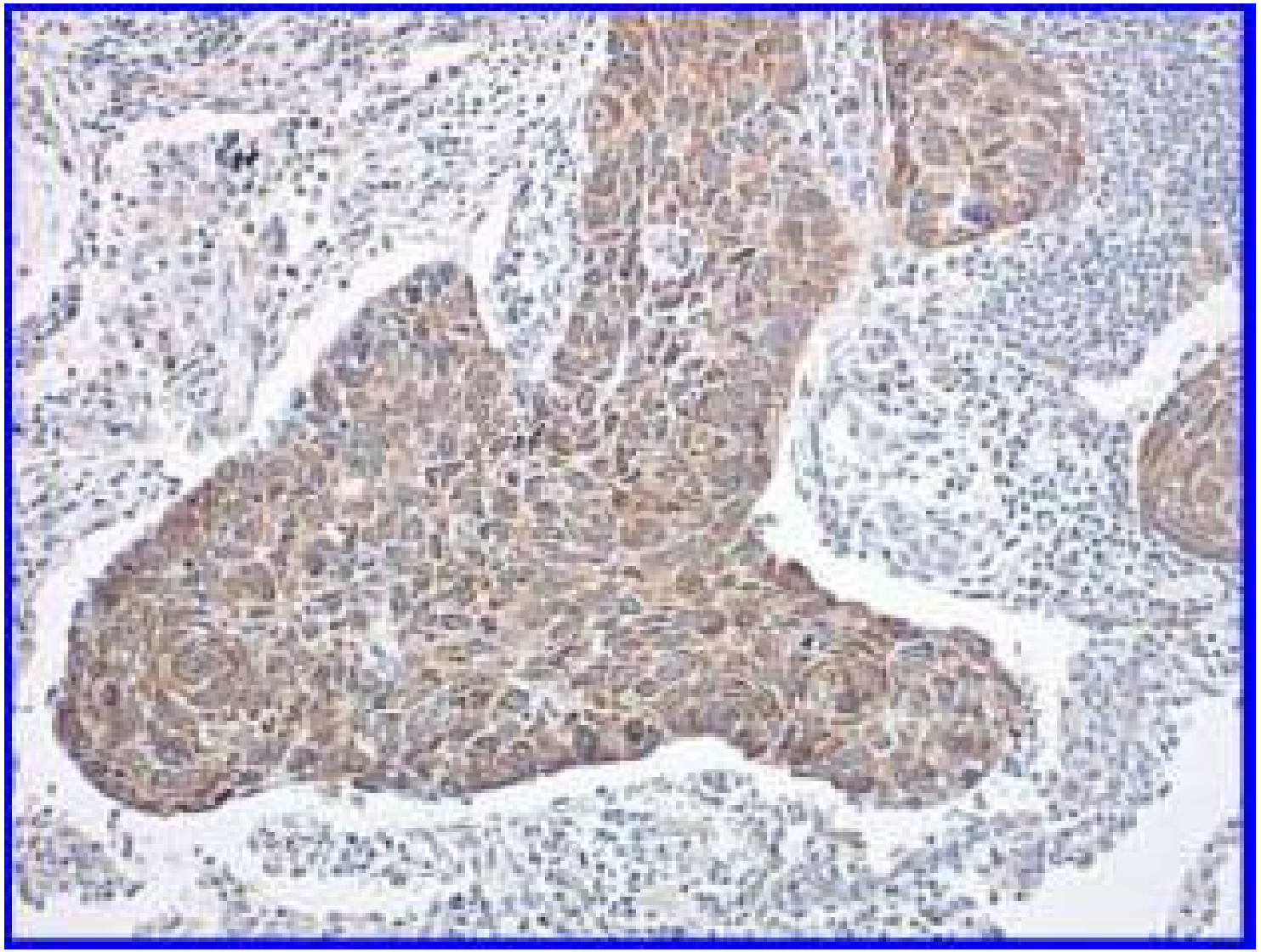
L523S – Background

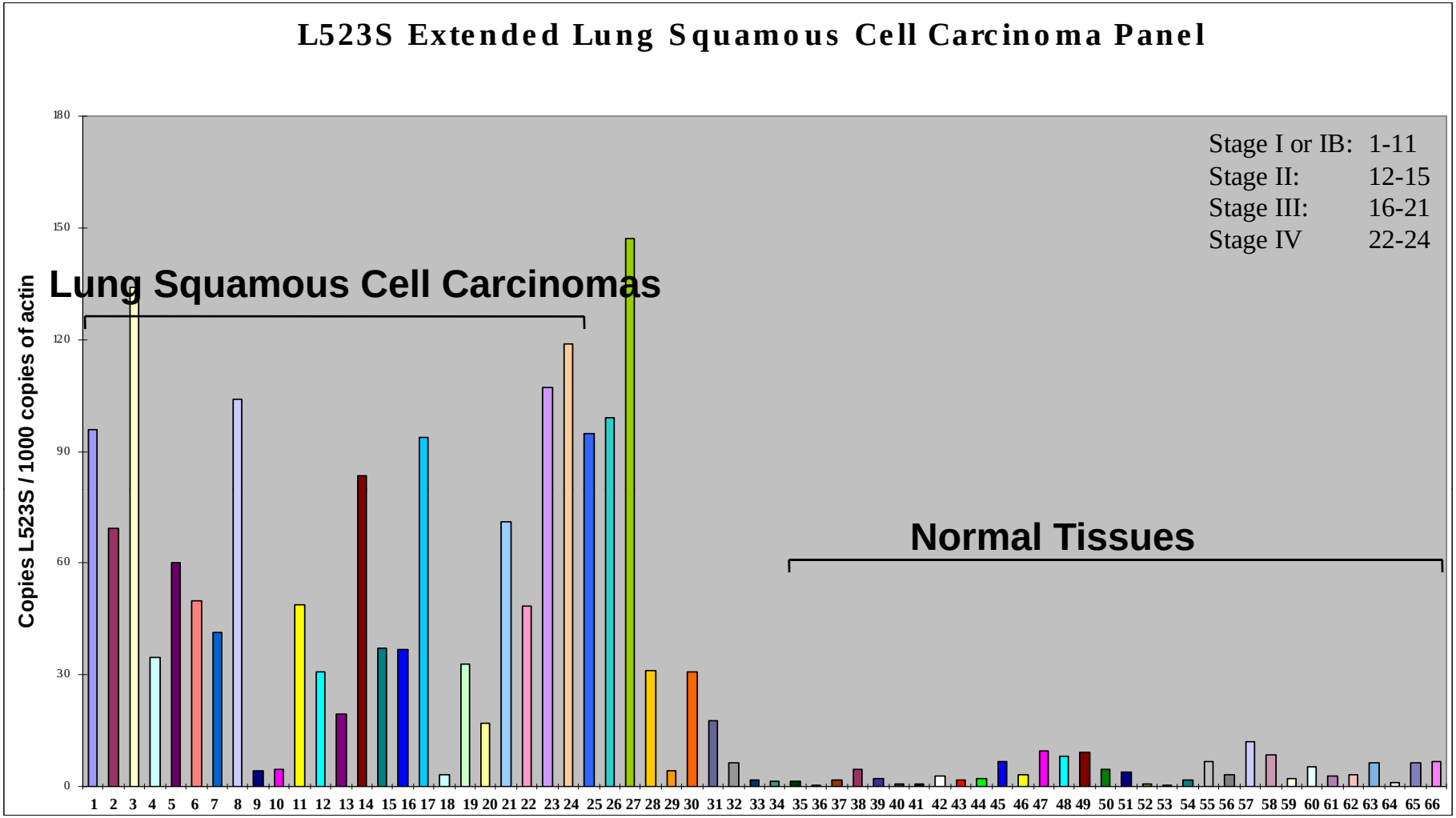
- **L523S – Non-Small Cell Lung Cancer (NSCLC) antigen**
  - **Identified by subtractive hybridization and cDNA microarray analysis.**
  - **Distribution**
    - **Assessed by Real Time RT-PCR**
    - **Validated by Immunohistochemistry**

- **Known gene**
  - **Encodes the KOC RNA binding protein.**
  - **Originally identified by differential expression screening of pancreatic cancer.**
  - **Over-expressed in pancreas cancer and in multiple tissues during embryo genesis.**

L523S – IHC: Squamous Cell Lung Cancer



L523S Extended Lung Squamous Cell Carcinoma Panel



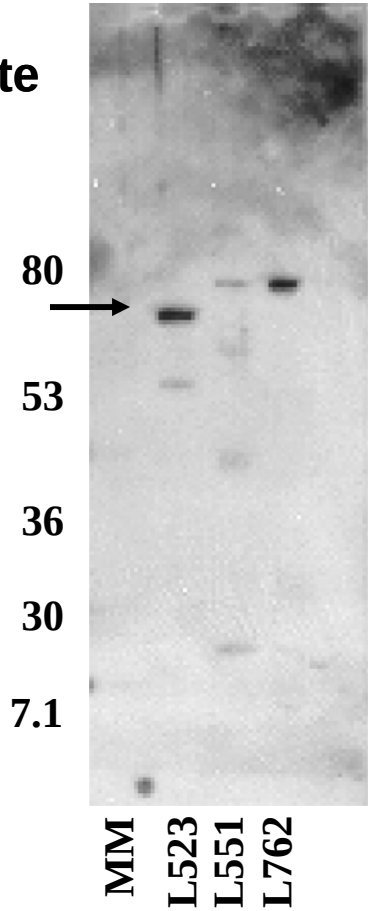
1. Squam. T – 507A
2. Squam. T – 510A
3. Squam. T – 741A
4. Squam. T – 744A
5. Squam. T – 745A
6. Squam. T – 746A
7. Squam. T – 824A
8. Squam. T – 835A
9. Squam. T – 841A
10. Squam. T – 1031A
11. Squam. T – 1037A1
12. Squam. T – 827A
13. Squam. T – 836A
14. Squam. T – 839A
15. Squam. T – 1010A
16. Squam. T – 96A
17. Squam. T – 509A
18. Squam. T – 742A
19. Squam. T – 743A
20. Squam. T – 842A
21. Squam. T – 1028A
22. Squam. T – 1036A
23. Squam. Scid LT46-90
24. Squam. Scid LT86-40
25. Head & Neck T – HN9
26. Head & Neck T – HN12
27. Adeno. T – 86-66
28. Adeno. T – LT86-17
29. Large cell T - 1007A
30. Large cell T - 1011A
31. Small cell T - 573A
32. Neur. Carcinoid 512A
33. Lung - 568A
34. Lung - 809A
35. Adrenal Gland - CT57
36. Bladder - INV 8905048
37. Brain - CT 8090440
38. Brain - CT 42
39. Bone Marrow - CT 003x
40. Bronchus - 557A
41. Colon - 670A
42. Esophagus - INV 204x
43. Heart - 560A
44. Kidney - 551A
45. Kidney - 1054A
46. Liver - 558A
47. Liver - CT 8120082
48. Lymph Node - CT 91x
49. Pancreas - 321A
50. Pancreas - 586A
51. Pituitary Gland - CT60x
52. PBMC resting 721-9A
53. PBMC T cell - 724A
54. PBMC B cell - 737A
55. Salivary Gland - CT 60
56. Sk. Muscle - CT 81207
57. Sk. Muscle - CT 61202
58. Skin - INV 8911140
59. Small Intestine - CT 80
60. Soft Pallet - DV
61. Spleen - 163A
62. Stomach - 825A
63. Tonsil - DV
64. Thymus - SPAAm5
65. Thyroid Gland - CT 708
66. Trachea - 776A

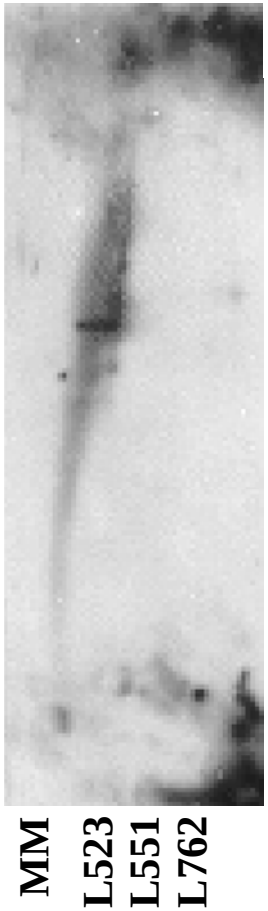
Endogenous expression of L523S

- **Low-level expression: ovary, fallopian tube, colon, bronchus, tonsil, gallbladder, and pituitary gland**
- **Tumor expression is greater than normal tissue**
- **Expect beneficial therapeutic ratio**

Antibody of L523S in Lung Cancer Patient
(L532S Western blot of pleural effusion)

Without E. coli lysate





With E. coli lysate

Lung Cancer Patients: L523S Ab Responses

1. MNKLYIGNLSENAAPSDLESIFKDAKIPVSGPFLVKTGY **AFVDCPDESWA**
Peptide #5

51. **LKAIEALSG**KIELHGKPIEVEHSVPKRQR**IRKLQIRNIPPHLQWEVLDS**L
Peptide #9

101. LVQYGVVESCEQVNTDSETAVVNVTYSSKDQARQALDKLNGFQLENFTL**K**

151. **VAYIPDETA AQQNPLQQPR**GRRGLGQRGS SRQGSPGSVSKQKPCDLPLRL
Peptide #16

201. LVPTQFVGAIIGKEGATIRNITKQTQSKI **DVHRKENAGAAEKSITILST**P
Peptide #24

251. EGTSAACKSILEIMHKEAQDIKFTEEIPLKILAHNNFVGRLIGKEGRNLK

301. KIEQDTDTK**ITISPLQELT LYNPERTITVKGNVETCAKA EEEIMKKIRES**
Peptide #31/32 **Peptide #34**

351. **YENDIASMN**LQAHLIPGLN**LNALGLFPPTSGMPPPTSGP**PSAMTPPYPQF
Peptide #37

401. EQSETETVHLFIPALSVGAIIGKQGQHIKQLSRFAGASI **KIAPAEAPDAK**
Peptide #42

451. **VRMVIITGP**PEAQFKAQGRIYGKIKEENFVSPKEEVKLEAHIRVPSFAAG

501. RVIGKGGKTVNELQNLSSAEVVVPRDQTPDENDQVVVKITGHFYACQVAQ

551. RKIQEILTQ**VKQHQQQKALQSGPPQSRRK**
Peptide #53

Rationale for DNA/Adenovirus Vaccine

- **Background:**
  - **CD8+ CTL are critical**
  - **No "Off-the-shelf" regimen for CTL**
  - **Recombinant Adv induces strong responses, but to Adv antigens**
  - **Recombinant DNA elicits CTL, but weak responses**

- **Proposed Regimen:**
  - **Prime with rDNA**
    - **Elicit weak L523S CTL response**
    - **Skew response to L523S**
  - **Boost with rAdv**
    - **Augment weak L523S CTL response**
    - **Induced L523S Ab and helper T-cell response**

Limitations of Murine Models for Vaccine Therapy

- **Human Lung Cancer**
  - **Evolves over many years**
  - **Treated with curative-intent surgery**
    - **Minimal residual disease persists**
    - **Median time to recurrence ~18 months**

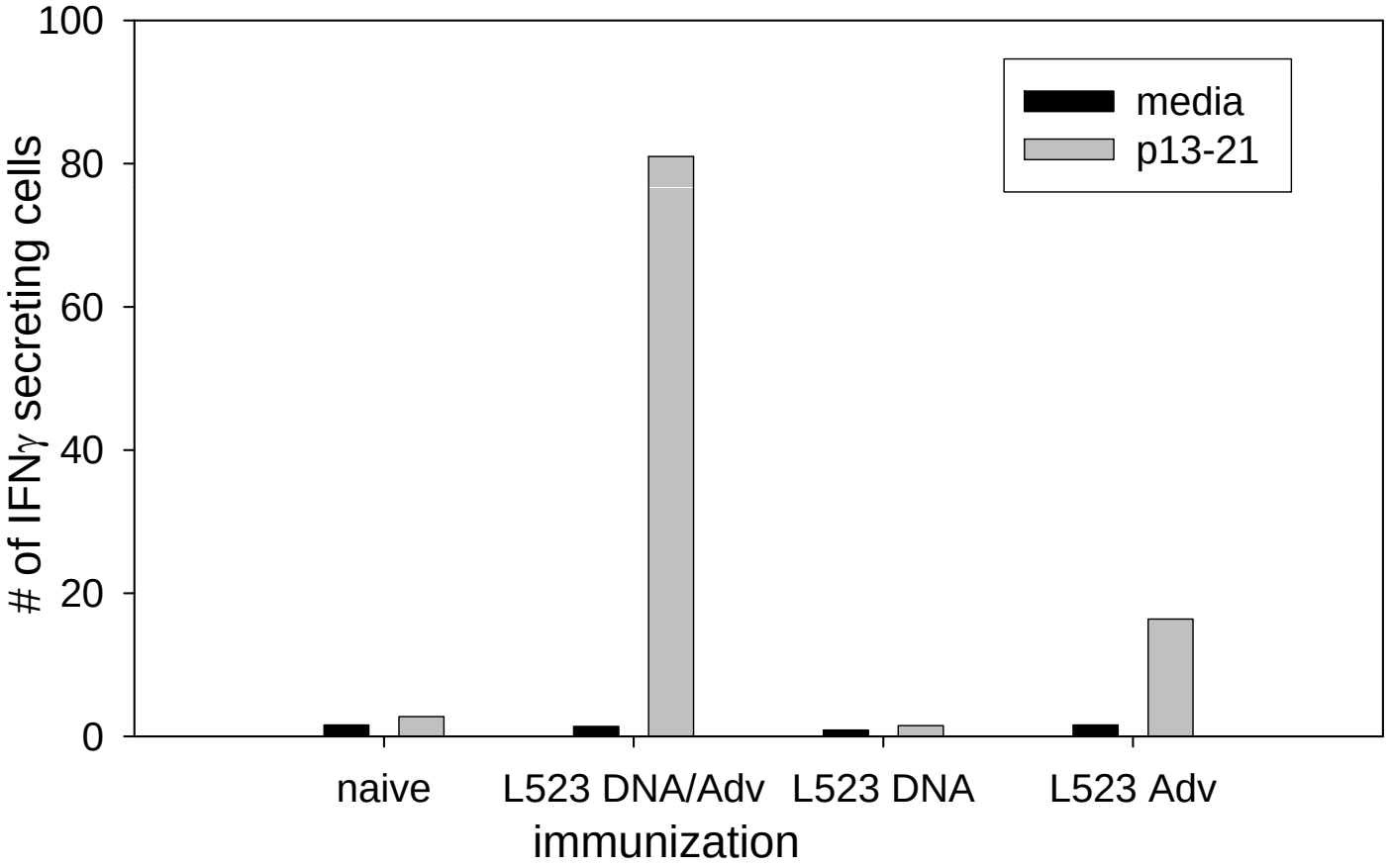
- **Murine Lung Cancer Models**
  - **Transplanted tumors**
  - **Different preclinical biology, growth rate, metastatic pattern, response to surgery, intrinsic antigenicity**
  - **Cannot replicate or approximate human NSCLC**

Role for Murine Vaccine Models

- **Assess immunogenicity of vaccine regimen**
  - **Cannot predict level of human immune response**
- **Assess toxicity**
  - **Can identify regimen related toxicity**
  - **Might identify autoimmune toxicity**
- **Cannot predict therapeutic outcome**

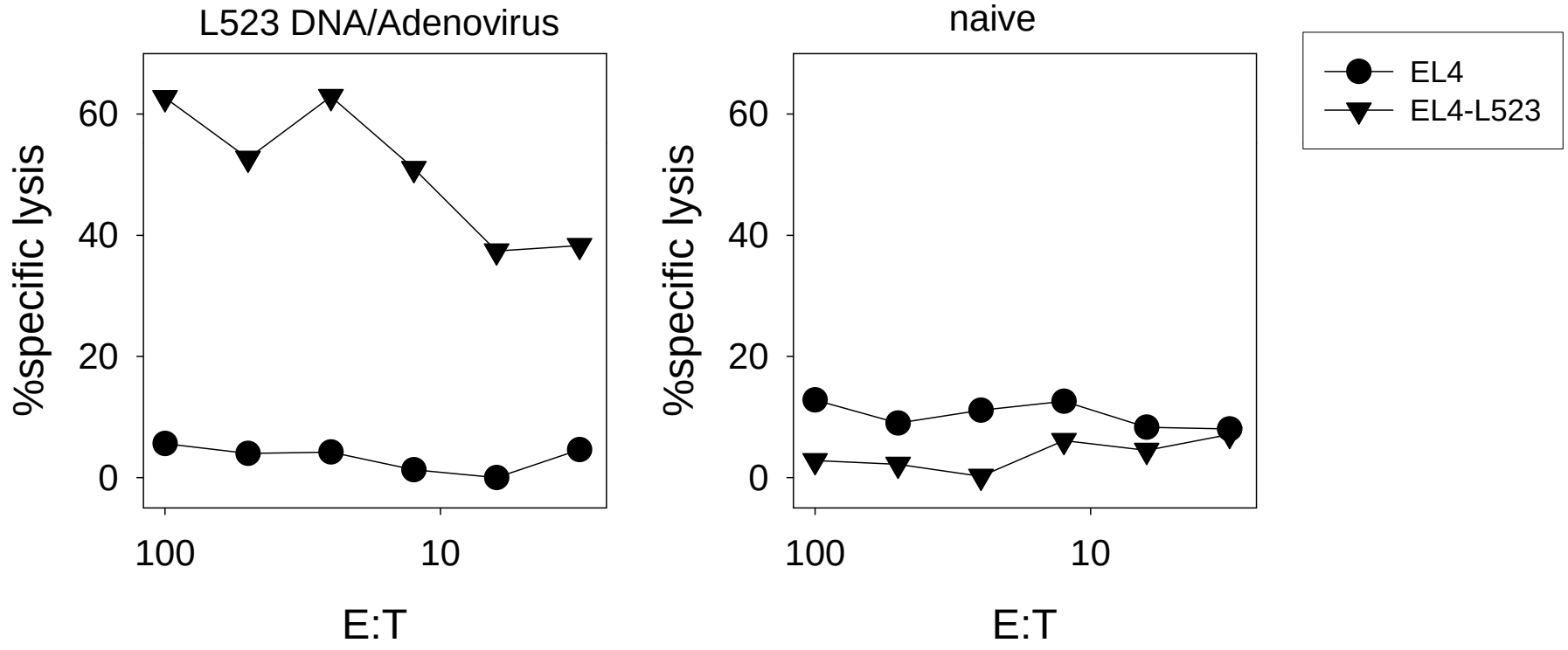
Immunogenicity of pVAX/L523S plus Ad/L523S Regimen

(IFNγ ELISPOT analysis - CD8+ T cells)

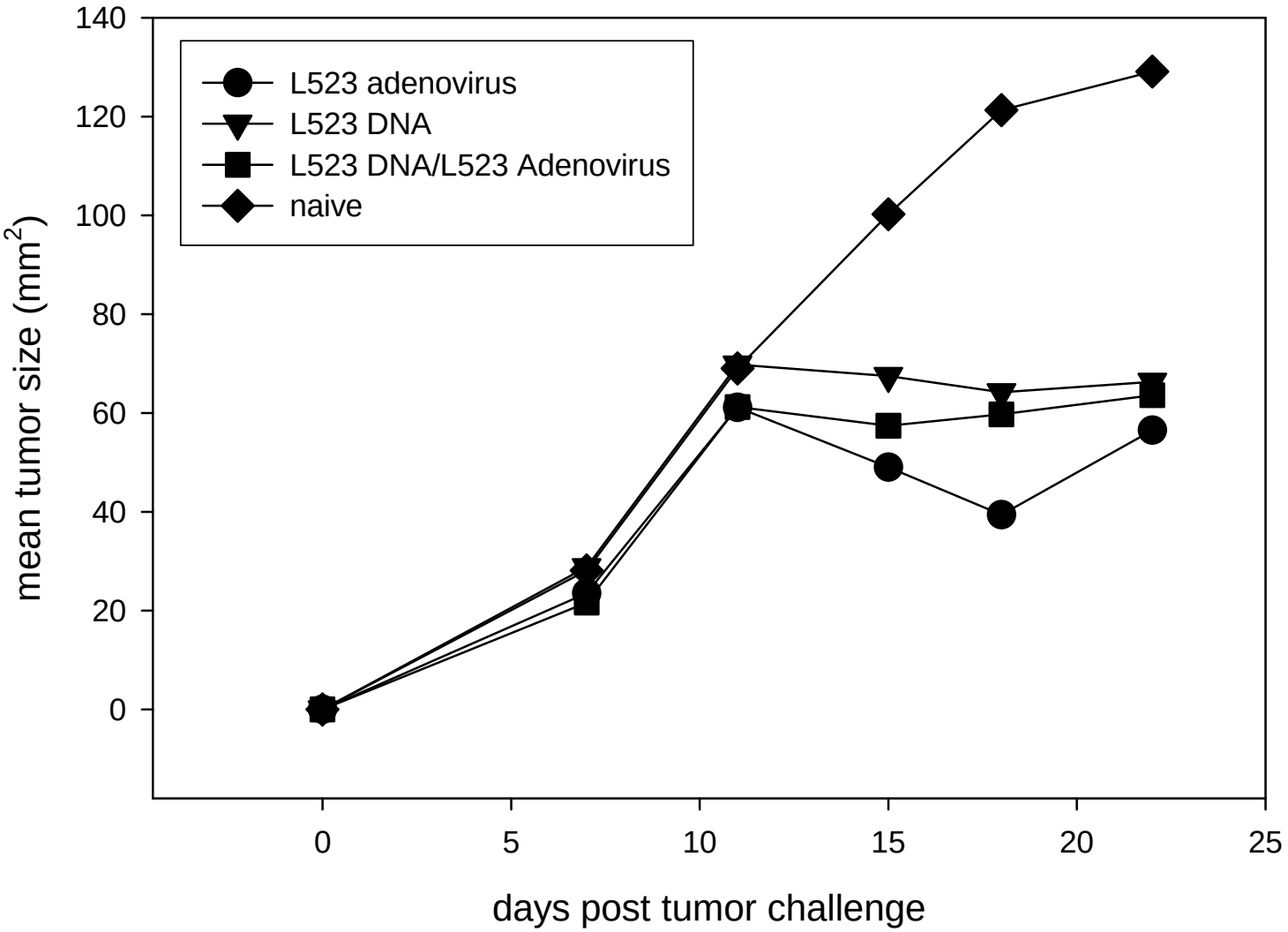


Immunogenicity of pVAX/L523S plus Ad/L523S Regimen

(Chromium Release Assay: Transduced Targets



Murine Tumor Protection
(EL-4/L523S Transduced Tumor)



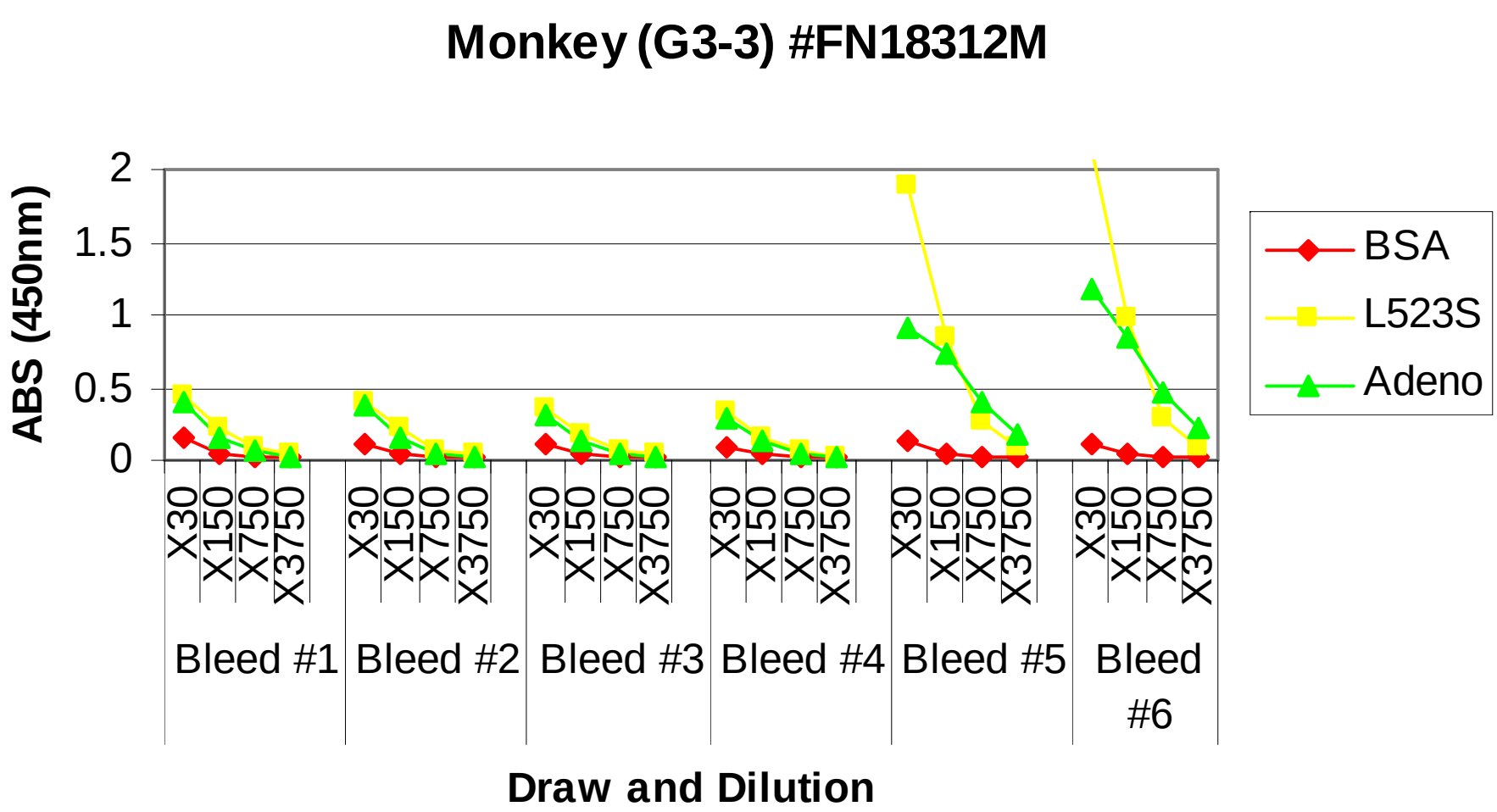
Summary of Cotton Rat & Primate Toxicity Studies

- Mild inflammation in the injection site by histopathology
- Mild reversible reactions in draining lymph nodes
  - Consistent with a strong immune response
- No other adverse clinical signs or adverse events
  - Serum chemistries, urinalysis, or other tests
- No inflammation or destruction of tissues with low-level L523S expression by histopathology

Validity of Cotton Rats & Primates for Toxicity Studies

- **Highly homologous L523S protein**
  - **99.3% homologous - Monkey**
  - **96.4% homologous - Mouse**
- **Equivalent tissue expression**
  - **Similar immunohistochemistry pattern**
- **Vaccine-induced immune response**

Primate Toxicity Study: Serum Ab



Animal Model Summary

- **pVAX/L523S & Ad/L523S are both**
  - **Immunogenic**
  - **Induce CTL**
- **pVAX/L523S plus Ad/L523S**
  - **Greater immune response than either alone**
- **No apparent untoward toxicity in Cotton Rats or Monkeys**
  - **Alone or**
  - **Together**

L523S – Protocol

- **Primary Objective**
  - **To evaluate the safety of the vaccine regimen administered as two priming doses of pVAX/L523S and two boosting doses of Ad/L523S**

- **Secondary Objectives:**
  - **To determine the immunogenicity**
    - **T-cell responses**
    - **Antibody responses**
    - **Effect of dose escalation on immunogenicity**

L523S – Protocol: Indication

- **Patients with Stage IB, IIA or IIB NSCLC**
  - **Undergone primary surgical resection within twelve months**
  - **No other therapy**
  - **No evidence of residual or recurrent disease**

Stage IB, IIA, or IIB NSCLC

- **Treated with primary surgical resection**
  - **>40% of patients with Stage I relapse and die**
  - **>60% of patients with Stage II relapse and die**

- **At relapse**
  - **Responds poorly to therapy**
  - **Incurable**
  - **Less than one year survival**

Target Population: Early Stage NSCLC

- **More immunogenic**
  - **Less cancer-induced immunosuppression**
  - **Less chemotherapy and radiation therapy-induced immunosuppression**

- **More effective**
  - **Higher effector-to-target ratio**
  - **Longer disease-free interval**

Classic Dose-Escalation Study Design

- 3 dose levels
- Cohort 1
  - 3 pts entered
  - No DLT – dose escalate
  - 1 DLT – add 3 pts
  - 1 more DLT – suspend trial
  - No more DLT – dose escalate Cohort 2
- Cohort 2 - repeat above
- MTD (Maximum Tolerated Dose)
  - One dose below cohort with >1 DLT
  - Might not be reached

Phase 1 Trial: Assessment of Safety

- **Standard hematological and biochemical parameters**
- **Physical examinations**
- **Specific Exams**
  - **Bronchus - chest x-rays and pulmonary function tests**
  - **Tonsil - physical exam**
  - **Colon - diarrhea**
  - **Gallbladder - liver function tests**
  - **Pituitary gland – TSH and free-T4**

Conclusions

- **Prior immune response is encouraging**
- **Prime-boost regimen with rDNA + rAdV is rational**
  - **Induces specific CTL**
  - **Benign toxicity studies**
- **Animal studies are appropriate and satisfactory for proceeding**
- **Early-stage NSCLC patients are appropriate**
- **Assays to assess safety are adequate**
- **Effect of overexpression of L523S protein is unknown, but minimal in toxicity studies**
- **Truncating the molecule is problematic**
- **Issues of consent and statistical analysis have been rectified**

Responses to Two Specific Issues

- **Possible Effects of L523S/Koc Overexpression**
- **Truncation of L523S**

Possible Effects of L523S/Koc Overexpression

- **Normal function:**
  - **Regulation of RNA stability and localization**
  - **Maps temporally and spatially in mouse embryos at different gestational stages**
  - **Putative target – IGF-II**

- **Function in Cancer:**
  - **Unknown**
  - **Might impact tumor cell proliferation by regulating post transcriptional or translational processes**

- **Mueller-Pillasch et al., Oncogene 1997 14(22):2729**
- **Mueller-Pillasch et al., Mech Dev 1999 88(1):95**
- **Nielsen et al., J., Mol. Cell Biol. 1999; 19:1262**

Possible Effects of L523S/Koc Overexpression

- **Transient expression**
  - **Likely**
  - **Toxicity studies**
    - **No evidence of morphologic changes at the site of injection**

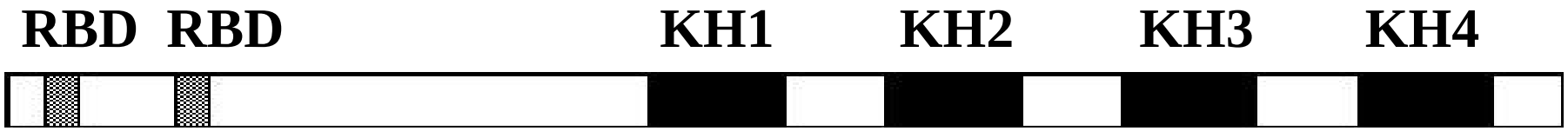
- **Constitutive expression**
  - **DNA and AdV – little or no evidence for integration**
  - **No data from L523S/Koc transgenic models**
  - **IGF-II is putative target (mitogenic factor)**
    - **Co-expressed RNA binding proteins repress expression of IGF-II**
  - **No data concerning other possible targets**

Truncation of L523S

- **Might decrease immunogenicity**
  - **Decrease immunogenic epitopes**
  - **Alter processing or incorporate new epitopes**

- **Might not abrogate function**
  - **Contains six RNA binding regions**
  - **Two types of RNA binding motifs**
    - **RNA recognition motif (RRM)**
    - **hnRNP K homology (KH) motif**

Schematic of L523S/Koc



RBD1: 4-9aa
RBD2: 31-45aa
KH1: 199-238aa
KH2: 280-311aa
KH3: 409-458aa
KH4: 491-541aa