Use of Allogeneic Tumor Vaccines Expressing the α(1,3)Galactosyltransferase Gene

Protocol 550: Breast Cancer

Protocol 552: Lung Cancer

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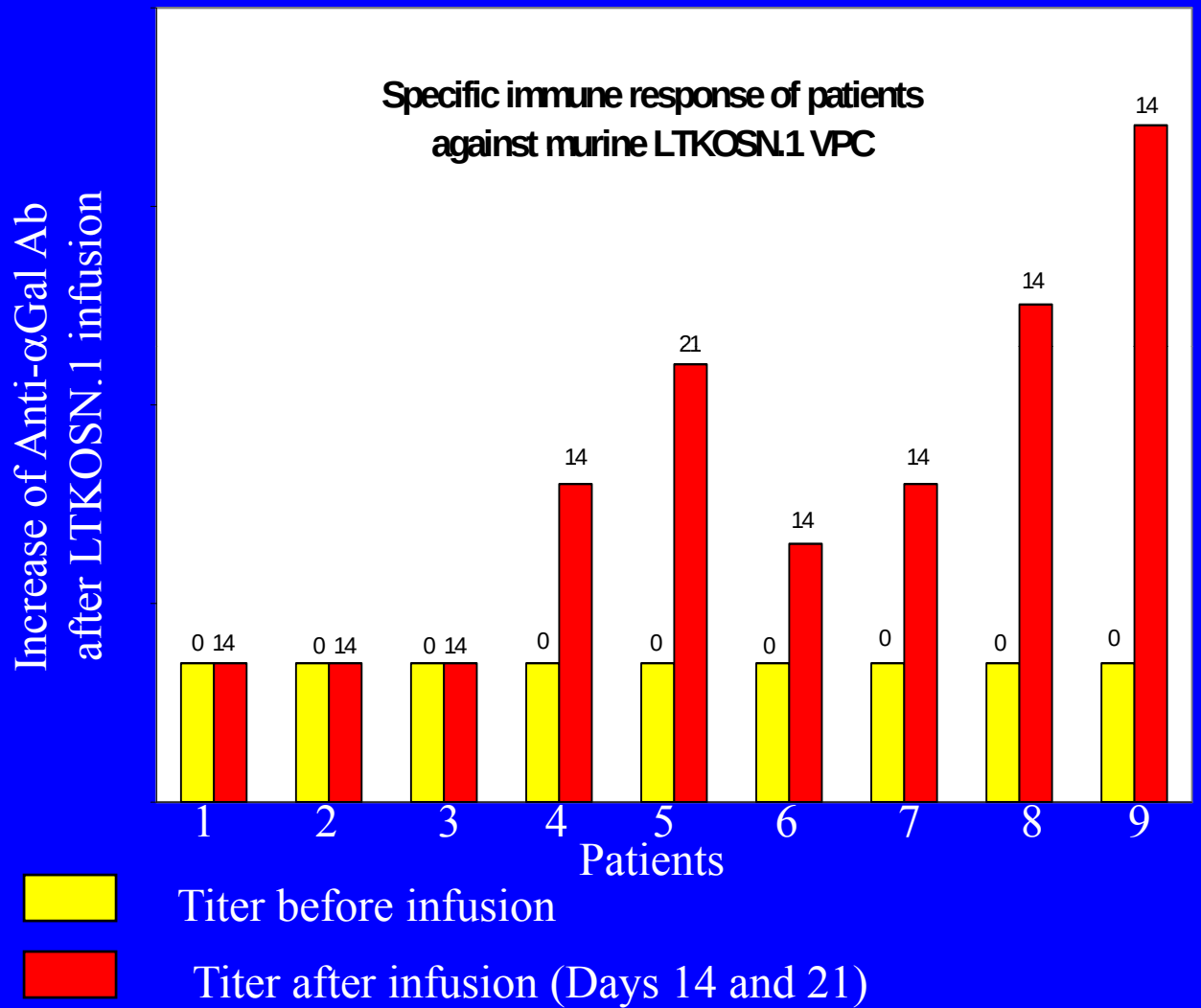
Xenotransplantation of Murine HSVtk Vector Producer Cells (VPC) into Women with Ovarian Cancer

- Failed chemotherapy with a platinum agent and paclitaxel
- ECOG performance status ≤ 2
- Patients received up to 7 billion murine cells by IP infusion
- No significant gene transfer, but some clinical responses including a CR by CT scan

Experimental and Clinical Data to Support Hyperacute Cancer Vaccine Approach

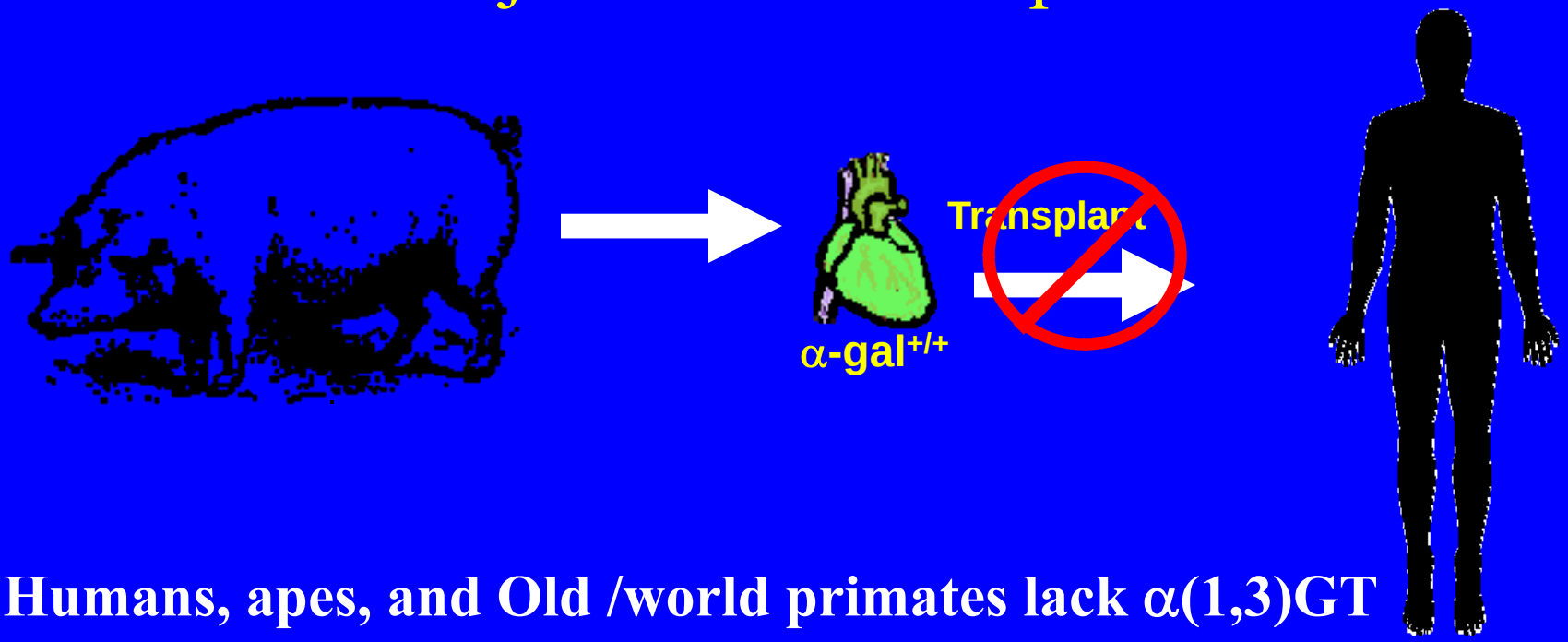
Patient	Age/ Stage	Tumor	Dose level	Dose (VPC)	Gene Transfer Observed	Result	Comments
1	64 IIIC	Ovarian	1	56 million	Not Tested	Partial Response	Local tumor necrosis
2	47 IIIC	Ovarian	1	57 million	No	Mixed response	Resolved ascites before GCV Rx
3	59 IIIC	Ovarian	1	56 million	No	Progressive Disease	Deceased 15 months after treatment
4	51 IIIC	Ovarian	2	680 million	Yes	Progressive Disease	Deceased 5 months after treatment
5	62 IIIC	Ovarian	2	700 million	Not Tested	Progressive Disease	Deceased 6 months after treatment
6	66 IIIC	Fallopian	2	840 million	Yes	Minimal Response	Deceased 8 months after treatment
7	73 IV	Ovarian	3	7 billion	No	Progressive Disease	Receiving chemotherapy
8	60 IIIC	Ovarian	3	6.3 billion	No	Stable	CT scan without disease CA125 decreased 70%
9	63 IIIC	Ovarian	3	6.2 billion	Yes	Progressive Disease	Deceased 3 months after treatment

Ovarian Cancer Patients Treated with Murine VPC Develop Increased Anti-αgal Ab Titers



Can the Hyperacute Rejection Phenomena Increase Anti-tumor Response ?

Xenotransplantation: problem of hyperacute rejection of a transplant



Humans, apes, and Old /world primates lack α(1,3)GT

Humans have high titer anti- αgal Ab

Anti-αgal antibodies are responsible for hyperacute rejection of xenotransplants

αGT expression Confers Susceptibility to Lysis by Normal Human Serum

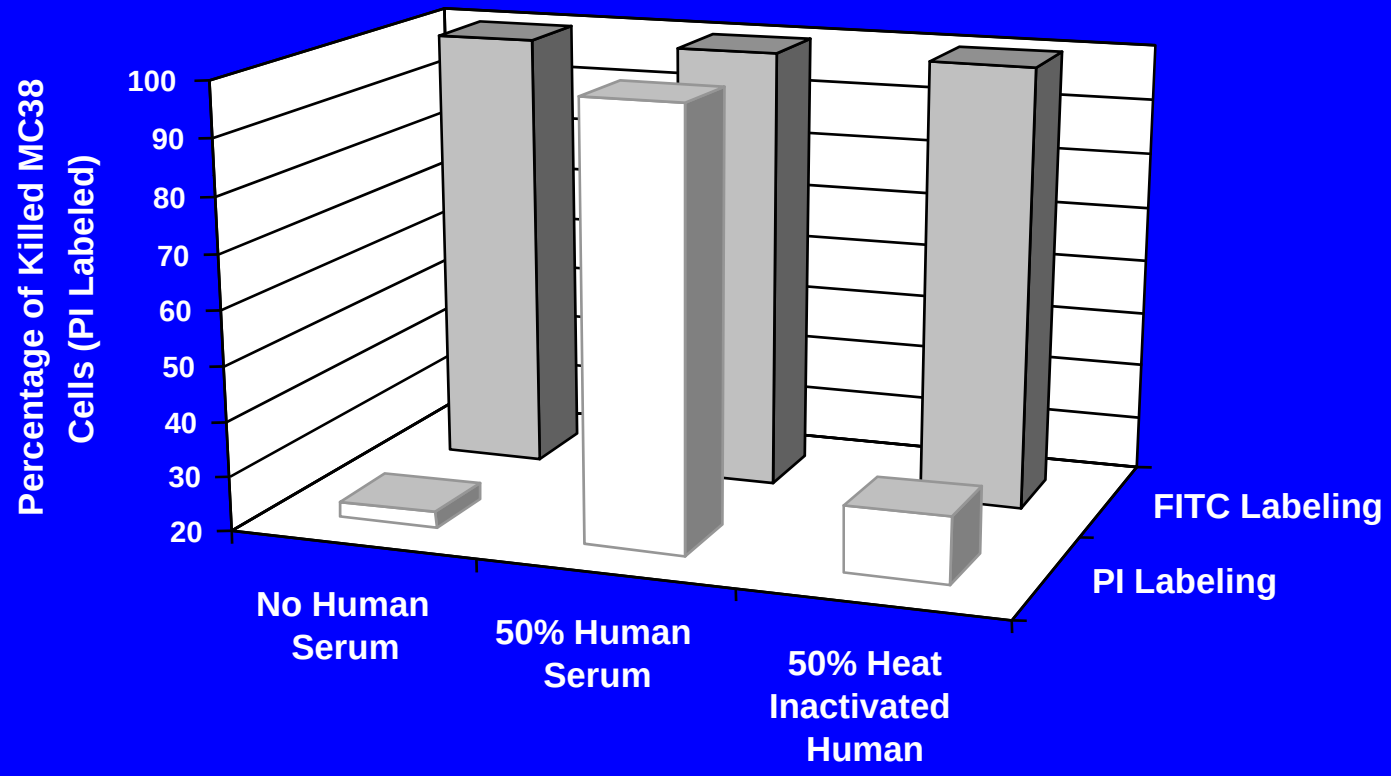
- Murine VPC and retroviral vectors are lysed by NHS secondary to anti- αgal Ab binding and activation of complement
- Human cancer cells transduced with α(1,3)GT gene express αgal and are lysed by NHS

	αgal Expression by FACS	Human Serum	Human Serum +sCR1	Human Serum Heat Inactivated
Cell Line		%Viable	% Viable	% Viable
A375	-	98.7	not done	96.9
A375aG.7	+	2.6	92	93.9
A375aG.8	+	11.1	91.6	95.5
A375aG.11	-	96.2	not done	not done

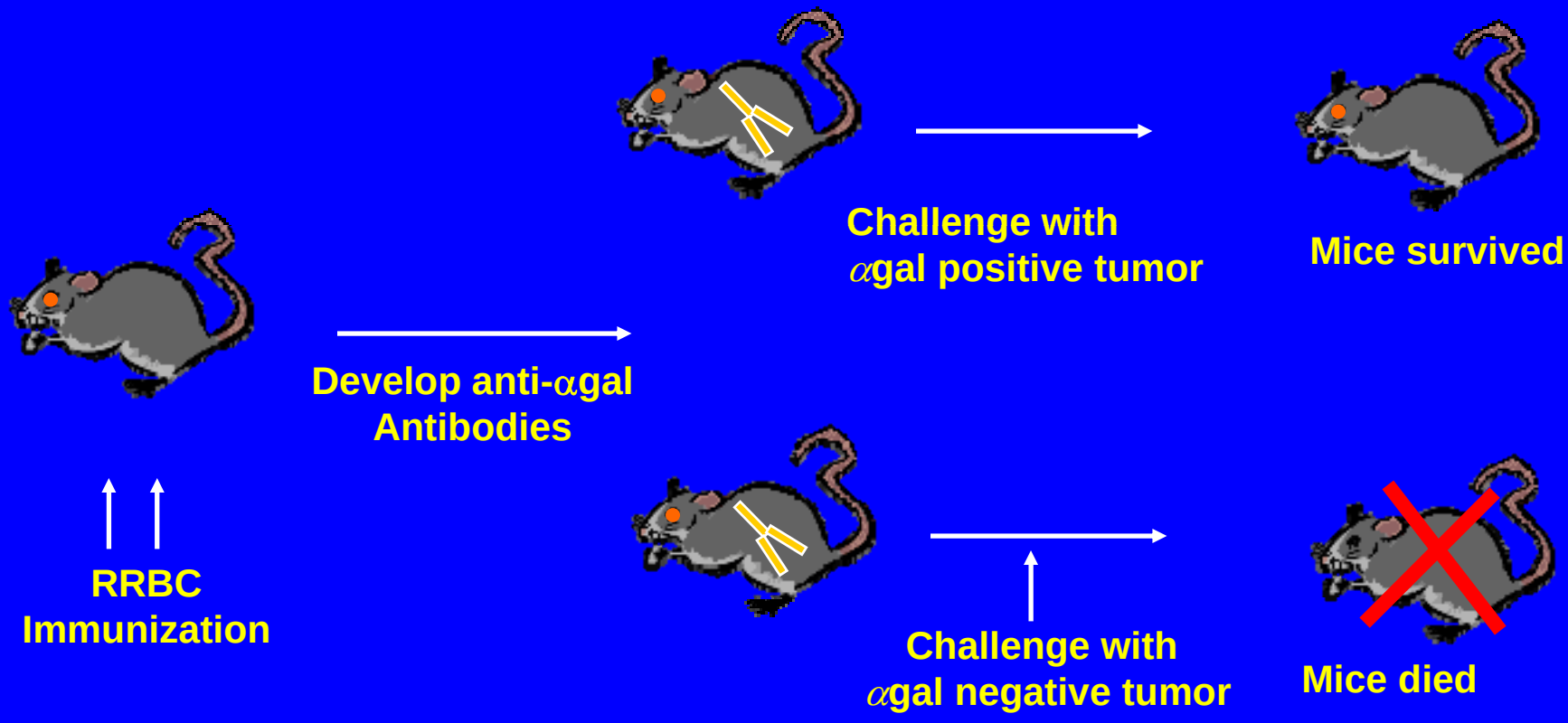
Preclinical Data: αGT KO Mouse Model

- The recently generated αGT "knock-out" (KO) mouse provides a small animal model to study the *in vivo* immune response against αgal epitopes on tumor cell lines.
- αGT-KO mice can be immunized to stimulate a high titer of anti-αgal antibody.

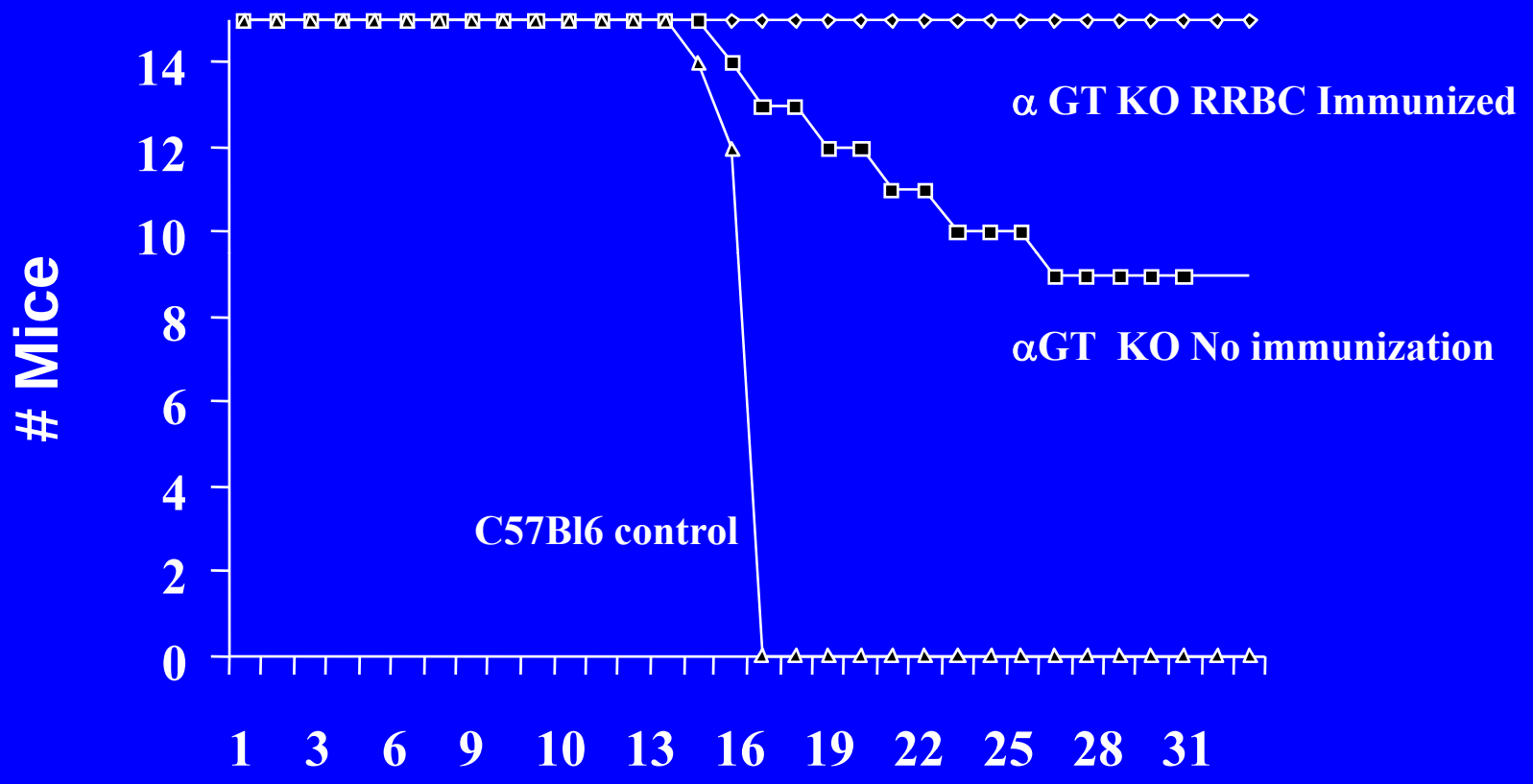
Killing of Murine MC38 Colon Cancer Cells With Normal Human Serum



In vivo effect of tumor cells expressing αgal in a murine tumor model

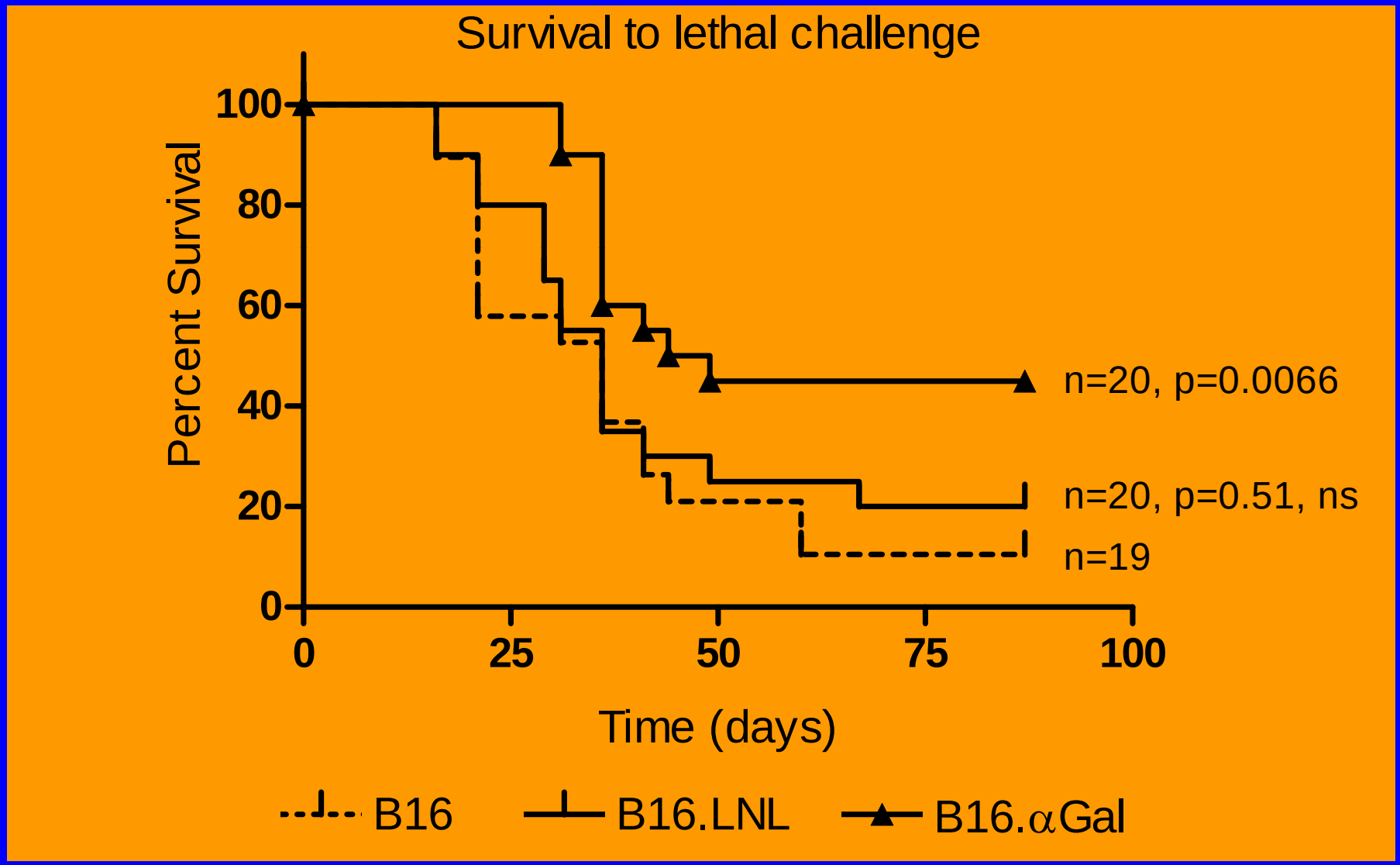


Challenge of Immunized Mice with αgal Positive MC38 Tumors

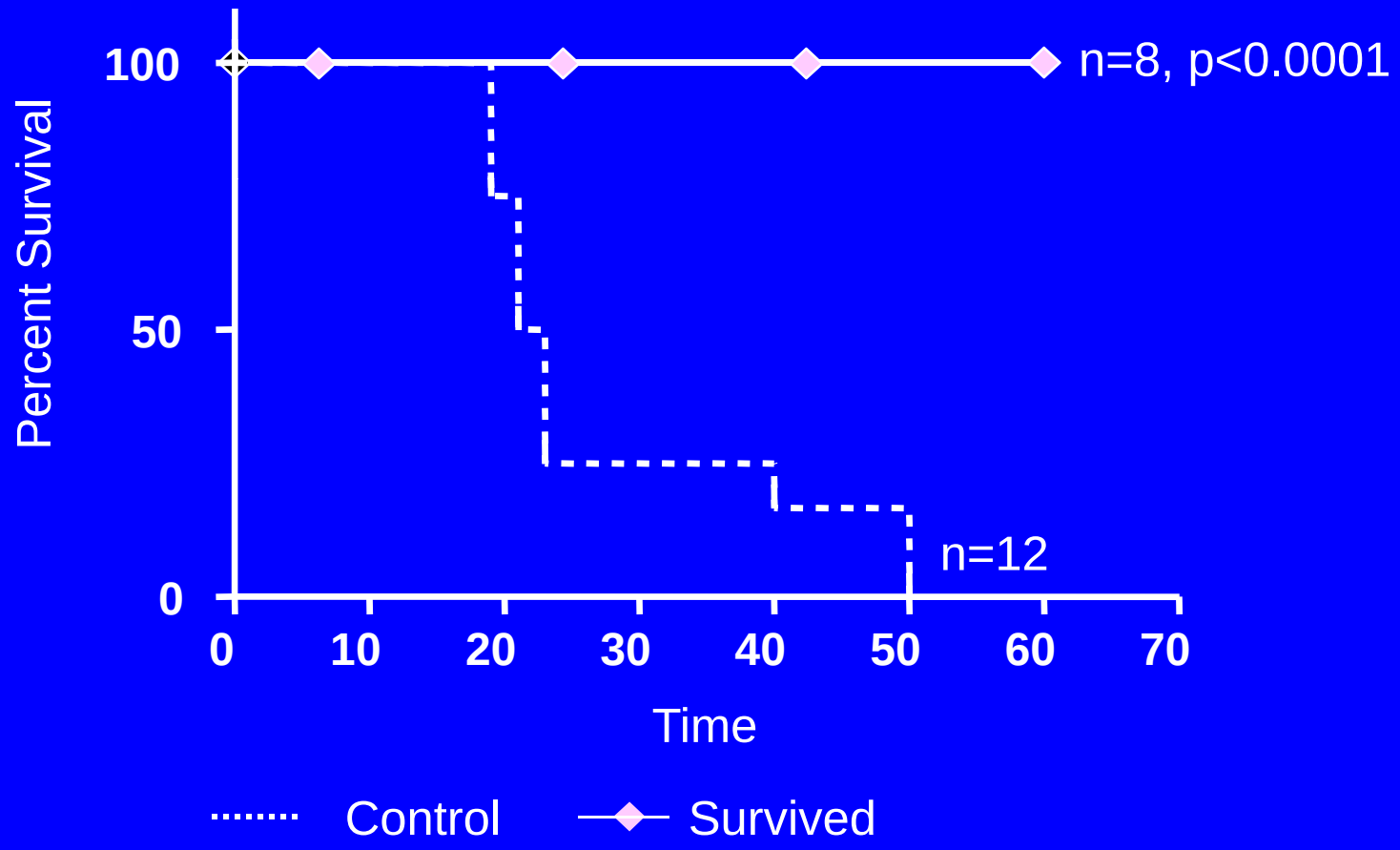


(Inoculating dose = 2.5 x 10^4 cells/mouse)

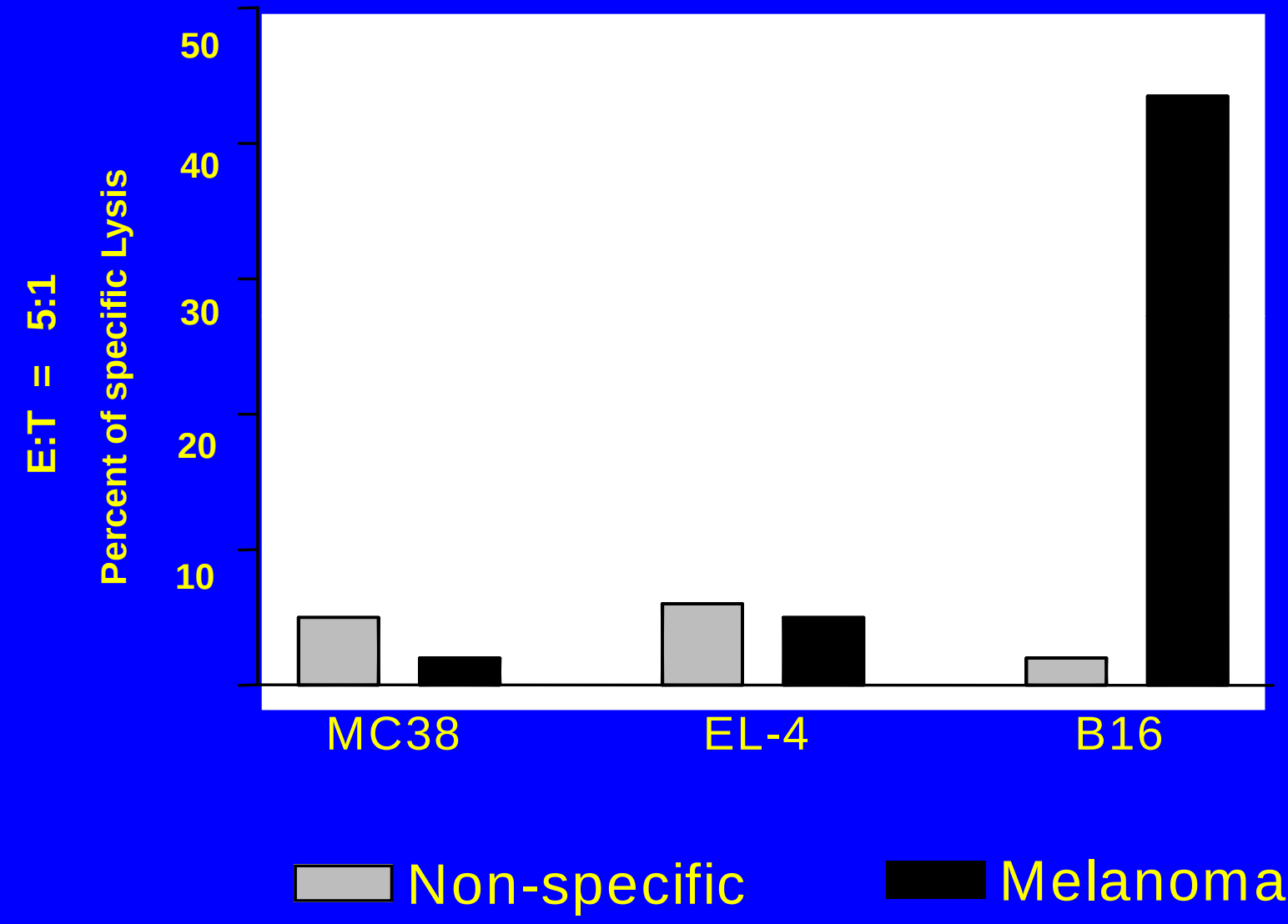
Survival of Immunized KO Mice Following B16 Tumor Challenge



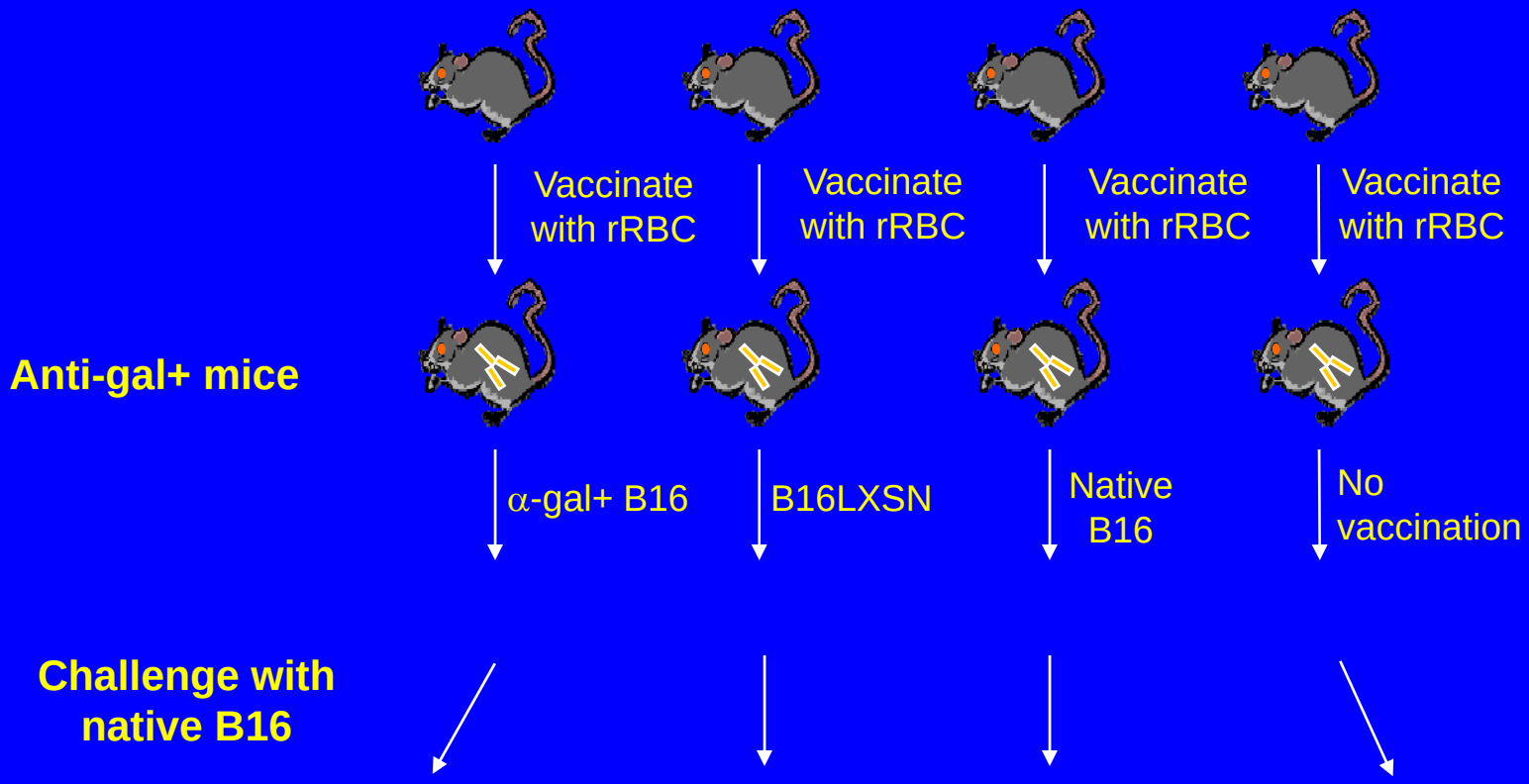
Immunity to αGal^{+} B16 Cells Rejects Challenge With αGal^{-} B16 Cells



Protected Mice Develop Melanoma Specific CTL Responsive to αgal Negative B16

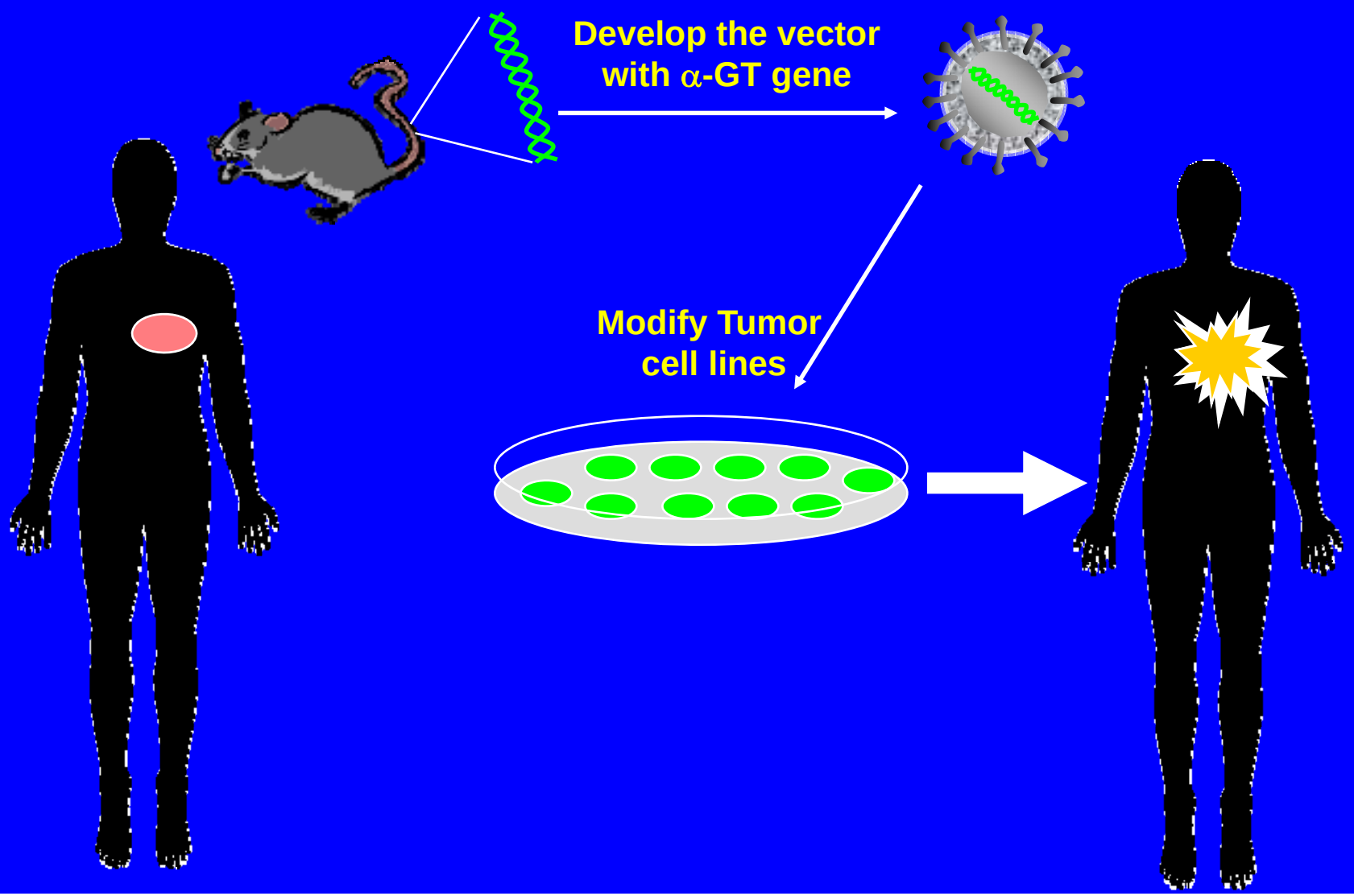


In vivo anti-tumor vaccination effects using tumor cells expressing α–gal

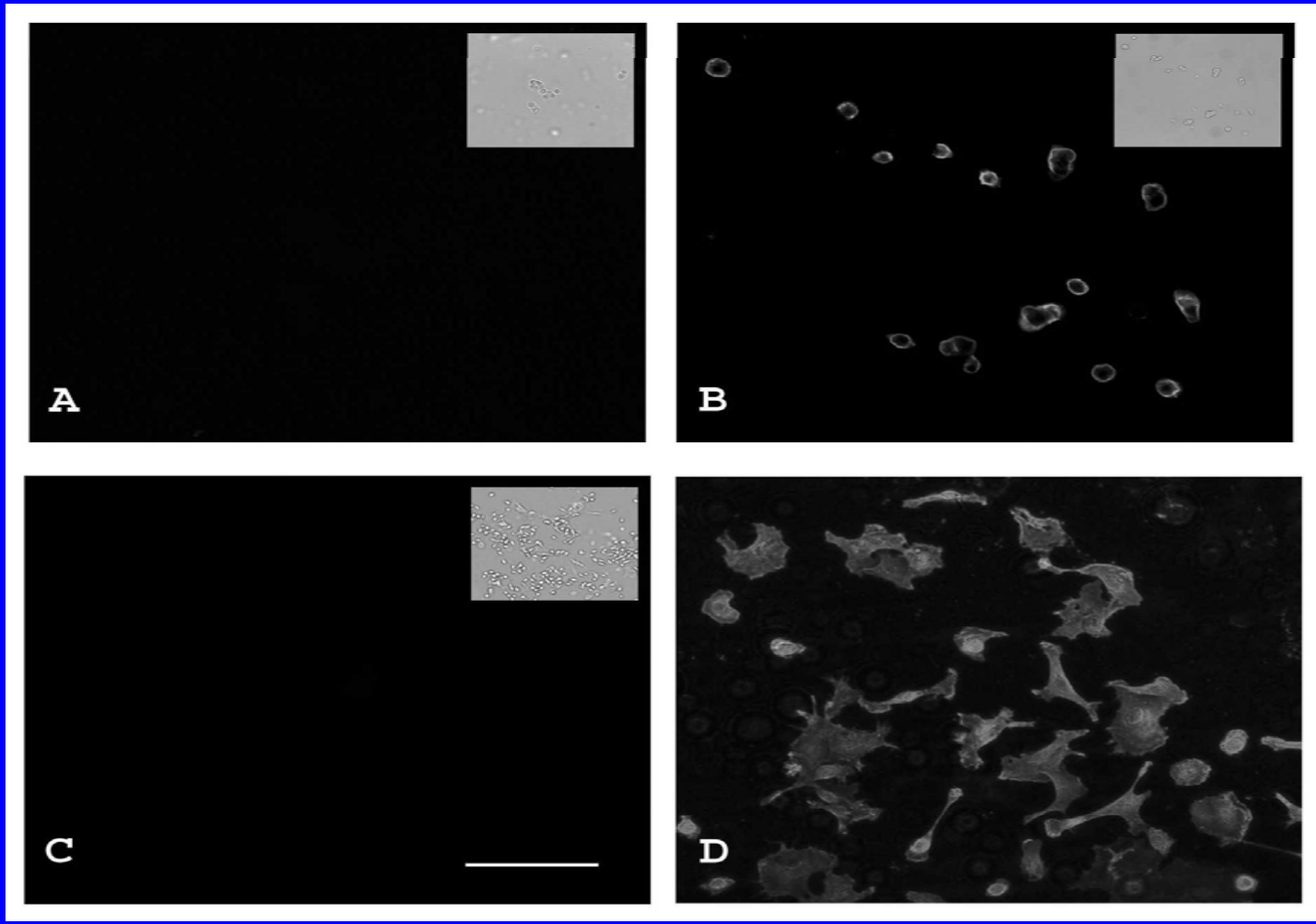


Tumor-free Survival at day 26 (12/04/02)			
12/20, p< 0.05	4/12, p> 0.05	4/14, p> 0.05	0/10

Hyperacute® Cancer Vaccine Using Gene Transfer



Human Breast Cancer Cells Expressing αGT Stained With Anti-αgal Ab (HAB-1 and HAB-2)

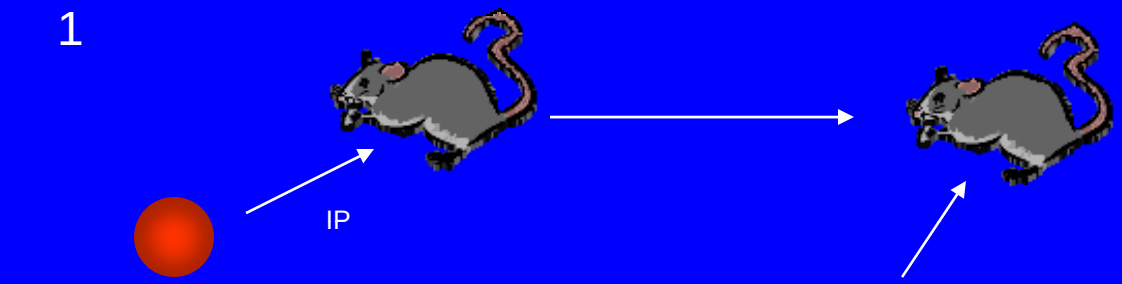


Toxicity Study for the Allogeneic Breast Cancer Vaccine

Animal treatments

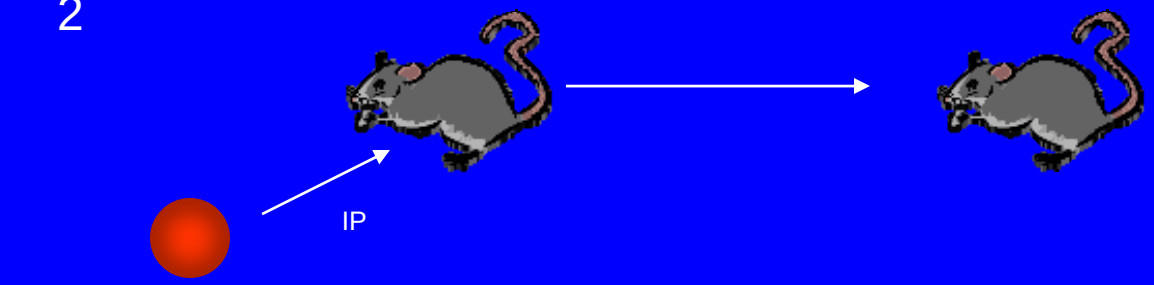
αGT KO mice
b/b Haplotype

1



Test Groups
2 weeks
4 weeks
6 weeks
6 months

2



RRBC Group

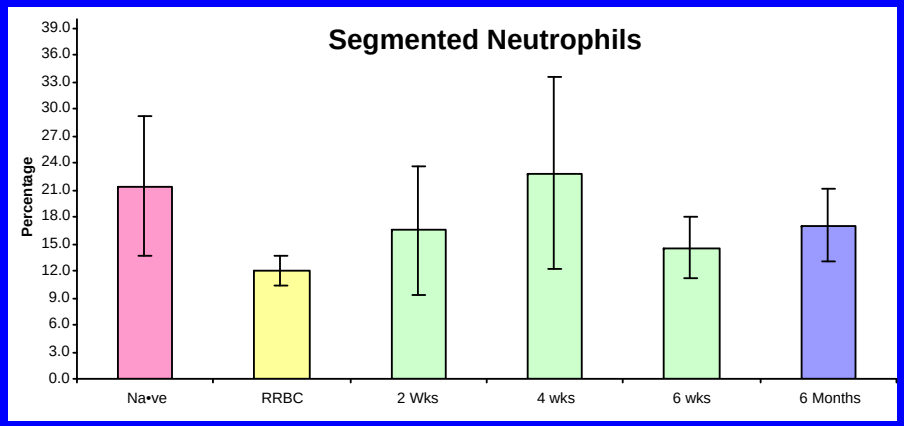
3

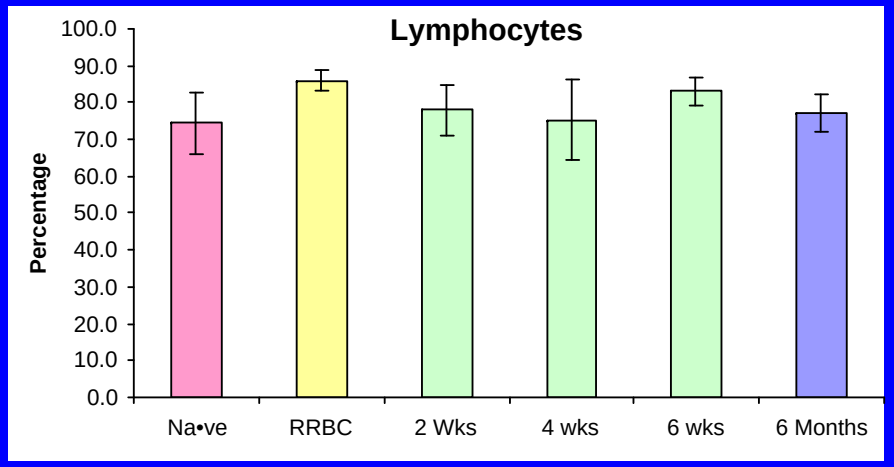
Naïve group

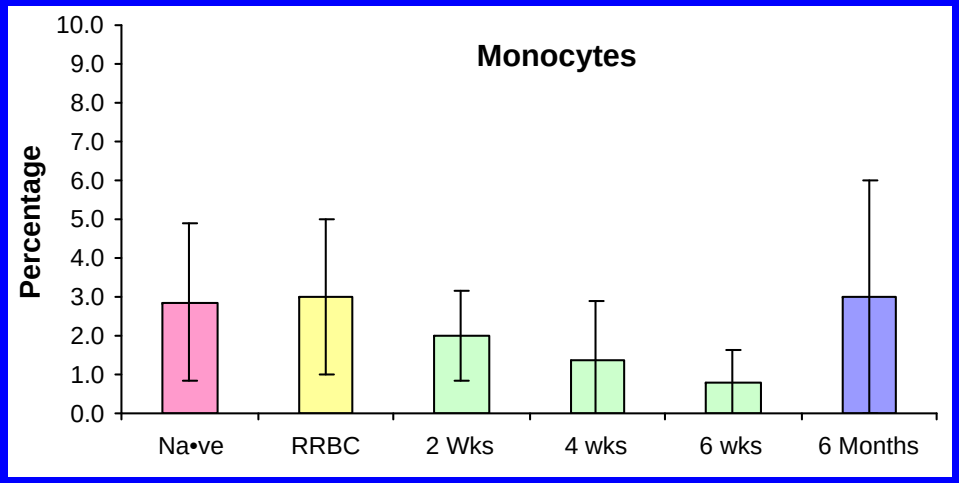
Absence of Immediate Hypersensitivity After Vaccination With Allogeneic Irradiated Breast Cancer Cells

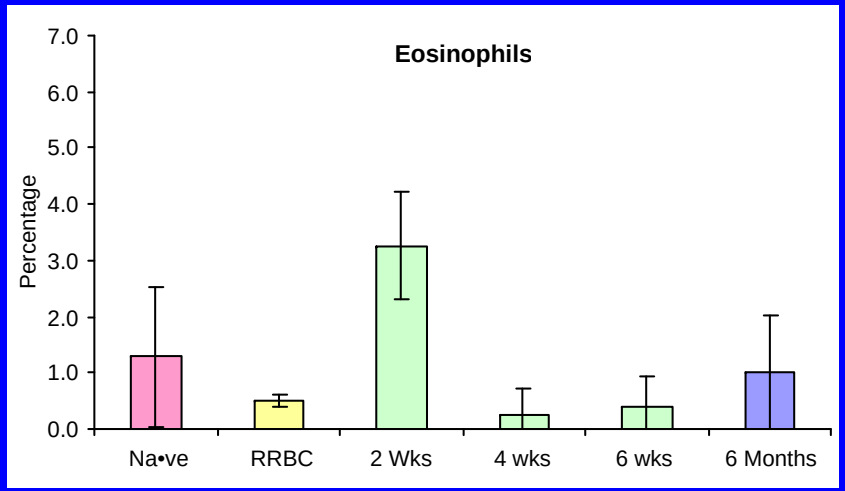
24 hr after vaccination

Hematologic Results









Animal Model Toxicity Study Summary:

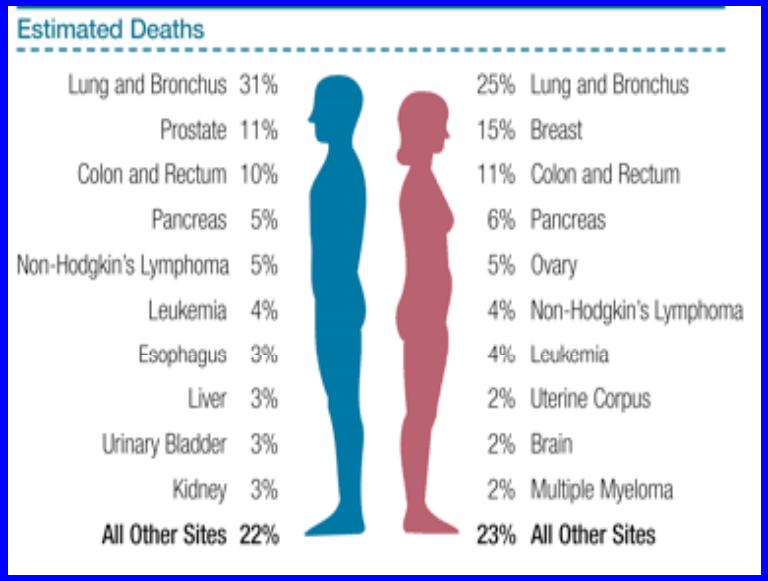
αGT KO mice (n=15) were studied after receiving EMT-6 allogeneic murine breast cancer cells (αGT^{+})

- No observed behavioral or motor abnormalities in mice
- No signs of immediate toxicity in the skin
- No significant hematologic alterations (n=15 vaccinated animals)
- Increase in Eosinophils, 2 weeks after vaccination (similar to observation in ovarian clinical trial patient receiving VPC)
- No pathology of major organ systems or mammary glands.

Toxicity in Phase I Trial of Murine VPC in Women With Ovarian Cancer

- Up to 7 billion αgal positive murine VPC administered
- No grade 3 or 4 toxicity observed
- Fever < 101.5 in most patients for 4 to 5 days
- Anorexia
- Mild to moderate nausea with or without vomiting
- Abdominal pain (mild to moderate) for up to 7 days

Lung Cancer

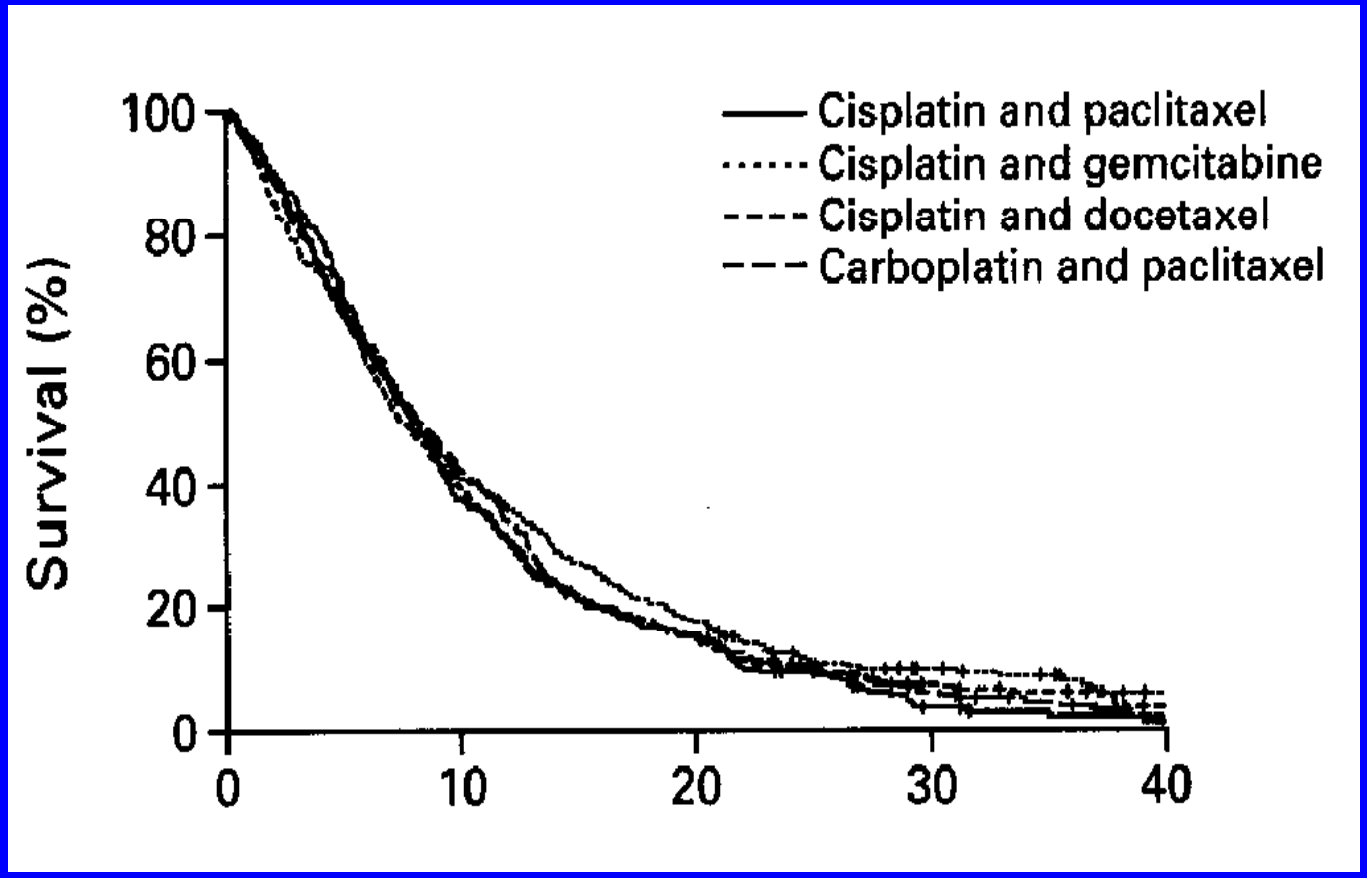


- U.S: 169,000 new cases and 155,000 deaths in 2002
- Leading cause of cancer death
- Surpassed breast as leading cause of cancer death in women in 1987
- ~15% of smokers develop lung cancer
- 5-year survival (all cases):
  - 1960- 10%
  - 2000- 13-15%

Advanced NSCLC

- 70% patients present with advanced stage (III/IV) disease
- Treated with chemotherapy± radiation
- 5-years survival:
  - Stage III- 10-19%
  - Stage IV- <2%

Chemotherapy in Advanced NSCLC



Study Objectives

- Phase I: Determine the side-effects, DLT and MTD of the HyperAcute™ Lung Cancer (HAL) Cell vaccine in patients with advanced or relapsed NSCLC
- Phase II: Determine the response of advanced or relapsed NSCLC to the HAL Cell vaccine
- To assess the immunological response of patients with advanced or relapsed NSCLC to the HAL Cell vaccine

Assessments

- Toxicity will be assessed using NCI CTC grading of symptoms, physical findings and laboratory tests
- Tumor response will be determined using NCI RECIST criteria for tumor measurements
- Determination of serum anti-a-gal titers, HAL cell stimulation of IFNg/IL-5 production by PBMC, and demonstration of CTL activity before and after completion of vaccination

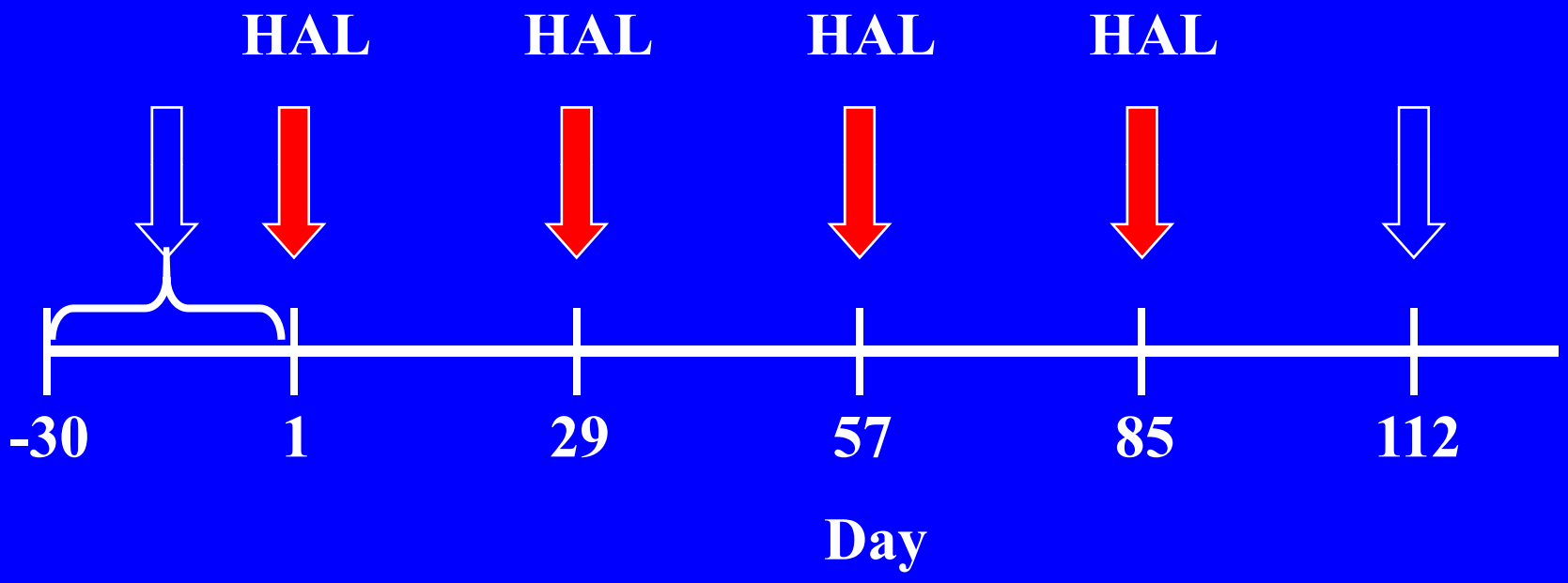
Eligibility Criteria

- NSCLC
- AJCC Stage IV, progressive or recurrent NSCLC
- ECOG PS ≤ 2
- Life expectancy ≥ 3 months
- Measurable disease
- Adequate organ function
- Up to 2 prior therapies
- Informed consent

Exclusion Criteria

- Age < 18 yrs.
- CNS involvement
- Hypercalcemia
- HIV, HCV or chronic active HBV
- Pregnant or nursing women
- Organ transplant or immunosuppressive therapy
- Autoimmune disease
- Active serious infection
- Other serious medical or psychiatric disease
- Known allergy to a(1,3)GT or vaccine cell lines

HyperAcute™ Lung Cancer Vaccine. Treatment Plan



HyperAcute™ Cell Vaccine NSCLC Trial Phase I Dose Escalation

Cohort	No. Pts.*	HAL Vaccine Cells (Total No. cells)
I	3	3×10^6
II	3	1×10^7
III	3	3×10^7
IV	3	1×10^8

*Cohort expanded to 6 Pts. if DLT seen.

Phase I/II Study of Antitumor Vaccination Using α(1,3)GT-Expressing Allogenic Tumor Cells in Patients with Breast Cancer

- ACS 2002 Breast Cancer Incidence 203,500 women with 39,600 deaths
- Tumor vaccine: allogeneic cells transfected by with the murine α(1,3)GT gene results in epitopes (αgal) glycoproteins and glycolipids.

Objectives

- Dose Limiting Toxicity (DLT)
- Maximum Tolerated Dose (MTD)
- Assess tumor response rate
- Assess immunologic response to antitumor vaccines with α(1,3)GT

Inclusion Criteria

- Histological diagnosis of recurrent breast cancer
- ECOG Performance Status ≤ 2
- Good end organ function
- Life expectancy ≥ 3 months
- Measurable or evaluable disease
- Failed one salvage regimen for stage IV disease
- Ability to provide Informed Consent

Exclusion Criteria

- Age < 18 yrs.
- CNS involvement
- Hypercalcemia
- HIV, HCV or chronic active HBV
- Pregnant or nursing women
- Organ transplant or immunosuppressive therapy
- Autoimmune disease
- Active serious infection
- Other serious medical or psychiatric disease
- Known allergy to $\alpha(1,3)$GT or vaccine cell lines

On-Study Testing

- CBC and metabolic profile
- CH50, ANA, RA, ESR
- Pregnancy test: β-HCG Flow cytometry: T & B cells, Tac (IL-2Rα), CD3, CD4, CD7, CD8, CD20, CD25
- Anti-α-gal antibodies
- Imaging studies of disease site(s)
- Skin test: tetanus, mumps, PPD-intermediate, candida albicans

Treatment

- HAB vaccine cells: intradermal injection on days 1, 29, 57, and 85
- Phase I: 3 patients at 3.0×10^6
  3 patients at 1.0×10^7
  3 patients at 3.0×10^7
  3 patients at 1.0×10^8
- Phase II: 32 patients at the maximum tolerated dose (MTD)

Correlative Studies

- Anti-α-gal antibody titers
- Cytolytic T-lymphocytes (CTL)
- Cytokine Assays: Interleukin-5 (IL-5), IL-10, and gamma interferon (IFN-γ)

Measurement of Effect

- RECIST Criteria for Solid Tumors
- Confirmation of Response at 4 weeks
- CT-Scan preferred for measurement