



Recombinant DNA Advisory Committee

GI-6301

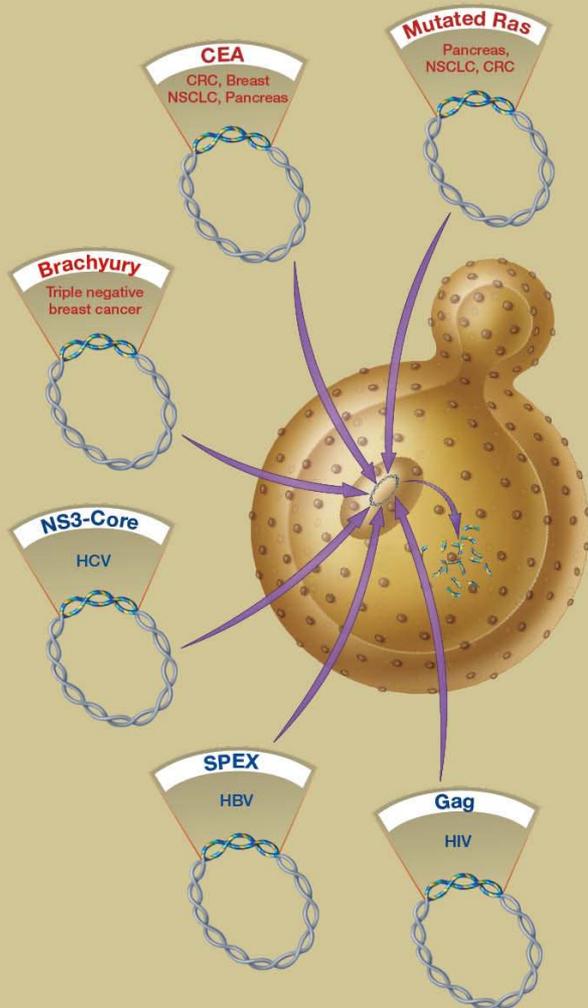
13 September 2011

# GlobeImmune



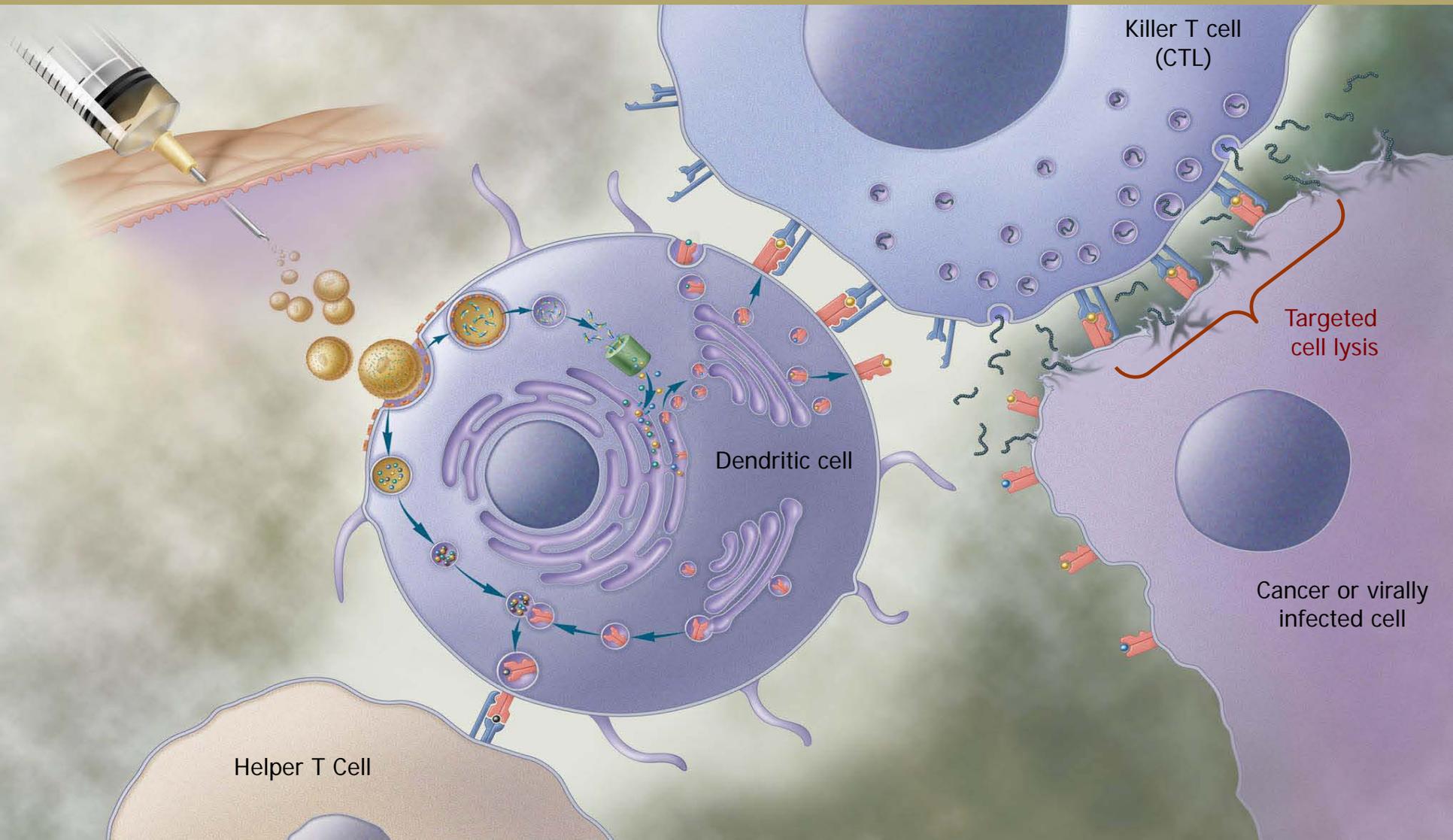
- Private company – 35 employees
- Three advanced clinical programs
  - GI-5005 - HCV – randomized phase 2 completed
  - GI-4000 – mutated Ras - phase 2
    - pancreas, lung and colon cancer
  - GI-6207 – CEA - phase 2
    - medullary thyroid cancer
- Long term NCI Tumor Immunology collaboration
  - GI-6207
  - GI-6301

# Tarmogen<sup>®</sup> platform



- Recombinant *S. cerevisiae*
- Selectively activates T cells
- Target to IND in 12-18 months
- Well tolerated
  - >300 subjects treated for up to 4 years
- Scalable manufacturing
  - Six products manufactured under GMP
  - Commercial scale is 250L

# Tarmogen mechanism of action



# Programs



INFECTIOUS DISEASES			ONCOLOGY				
PRODUCT	ANTIGEN	INDICATION	RESEARCH	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3
GI-5005	NS3/Core	Hepatitis C	[Green bar spanning Research, Preclinical, Phase 1, and Phase 2]				
GI-2010	Gag	HIV	[Green bar spanning Research and Preclinical]				
GI-13000	SPEX/SCORE	Hepatitis B	[Green bar in Research]				
GI-9000	Various	Fungal	[Green bar in Research]				
GI-4000	Mutated Ras	Pancreas, NSCLC, CRC	[Orange bar spanning Research, Preclinical, Phase 1, and Phase 2]				
GI-6207	CEA	Medullary thyroid cancer	[Orange bar spanning Research, Preclinical, and Phase 1]				
GI-6301	Brachyury	Triple negative breast cancer	[Orange bar spanning Research and Preclinical]				

# Clinical data



- GI-5005 HCV
  - Phase 1b - 52 treated subjects
    - Antigen specific cellular responses
    - ALT normalization
    - Viral load reductions
  - Phase 2b – 102 treated subjects
    - 20% relative improvement in sustained virologic response (SVR)
    - Greatest effect in genetically worst prognostic group
    - Normalization of T cell responses

# Clinical data



- **GI-4000**

- Phase 1 - 33 Ras mutation<sup>+</sup> CRC and pancreas cancer subjects
  - 90% Stage IV; average 3 prior lines of therapy
  - 90% with antigen-specific immune responses
- Phase 2
  - Pancreas cancer – 178 subjects / fully enrolled (randomized 1:1)
  - NSCLC – 24 subjects / MSKCC
    - Antigen specific cellular responses – ASCO 2011

- **GI-6207**

- Phase 1 – 25 subjects / NCI
  - Antigen specific cellular responses
  - Stable disease in 20% with advanced Stage IV disease – ASCO 2011

# Clinical platform safety (1)



- >300 subjects treated with Tarmogens at doses up to 40 YU for up to 4 years
  - GI-4000 dosing started June 2006
  - GI-5005 dosing started December 2007
- Phase 2 programs monitored by independent Data Safety and Monitoring Board (DSMB)
- No significant novel toxicities or dose limiting toxicities observed

# Clinical platform safety (2)

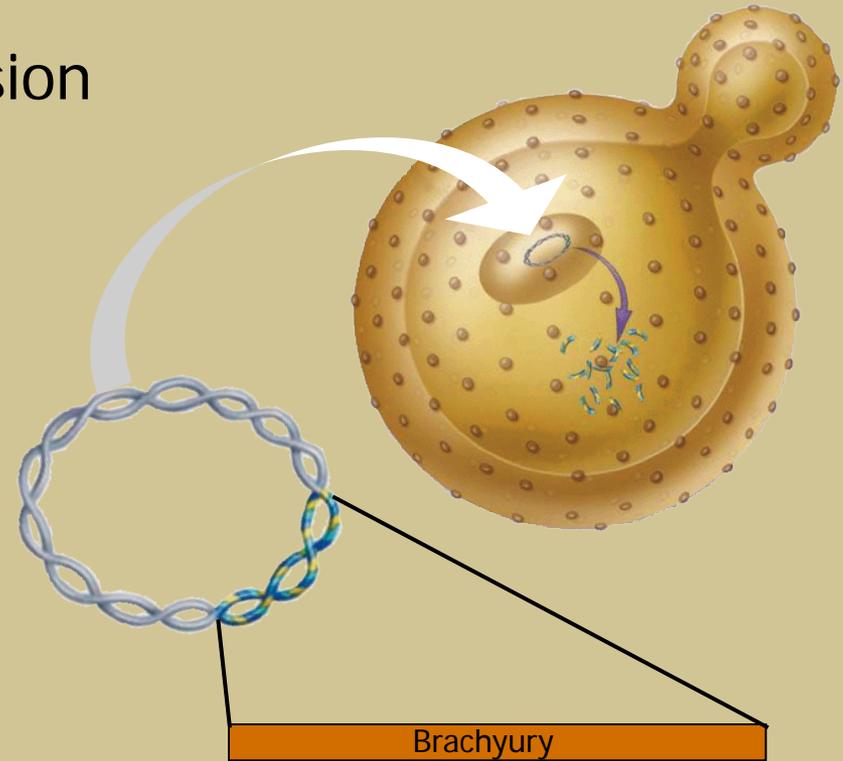


Product	Target	IND Number	Clinical Indication	Number of Subjects exposed	Safety
GI-4000	Mutated <i>ras</i>	BB-IND # 11653	Pancreas cancer, advanced colorectal cancer, non-small cell lung cancer	147 subjects	<ul style="list-style-type: none"> <li>Well tolerated with no significant safety findings</li> <li>Non-serious AEs limited to mild constitutional complaints and local injection site reactions.</li> <li>13 Data Safety and Monitoring Committee meetings held with no significant safety findings.</li> </ul>
GI-5005	NS3-core fusion protein	BB-IND # 12360	Subjects with genotype 1 chronic hepatitis C infection	154 subjects	<ul style="list-style-type: none"> <li>Well tolerated with no significant safety findings</li> <li>Evaluated by an independent data safety and monitoring board (DSMB) demonstrating excellent safety and tolerability profile</li> <li>No significant novel toxicities or dose limiting toxicities observed.</li> </ul>
GI-6207	CEA	BB-IND # 13934	Metastatic CEA-over-expressing cancers	25 subjects	<ul style="list-style-type: none"> <li>No dose-limiting toxicity observed.</li> <li>1 subject with pulmonary and pericardial metastasis prior to enrollment                             <ul style="list-style-type: none"> <li>Grade 3 toxicity (pleuritis and pericarditis) following vaccination</li> </ul> </li> </ul>

# GI-6301 - brachyury



- Key factor in metastatic progression
- Brachyury<sup>+</sup> cells resistant to chemotherapy and radiotherapy
- Highly expressed in
  - Lung, breast, ovary, colon, prostate, pancreas
- IND ~ YE 2011
- NCI sponsored phase 1





Recombinant DNA Advisory Committee

GI-6301

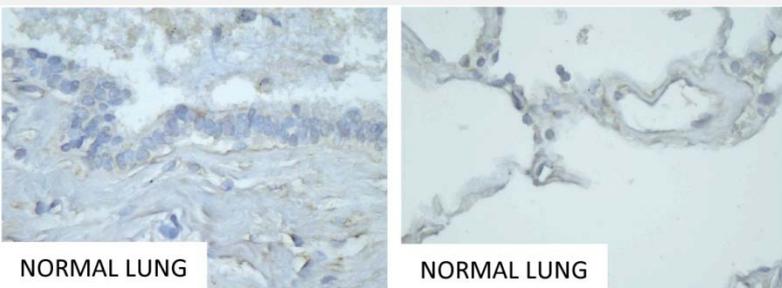
13 September 2011

# Immunohistochemistry Analysis of Brachyury Expression in Lung Tumor vs. Normal Tissues

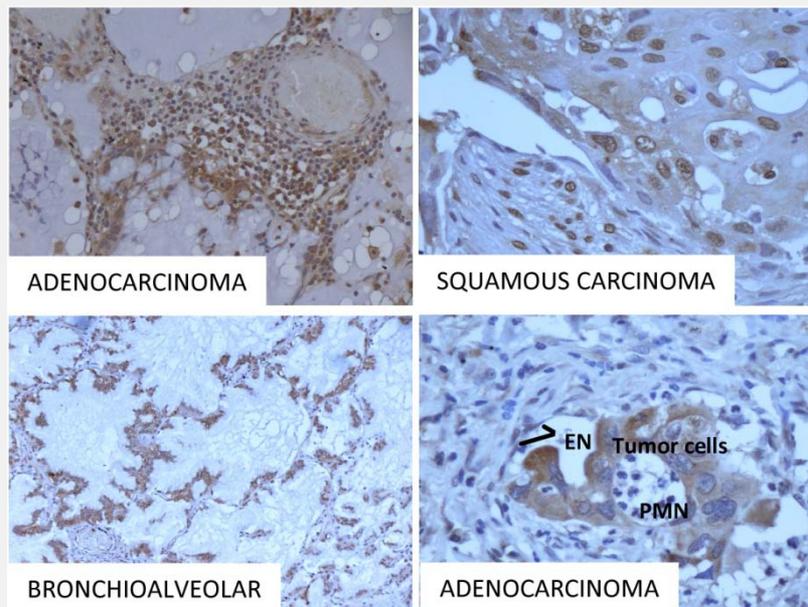
Collaboration with Drs. Fiorella Guadagni and Mario Roselli, Rome, Italy

Normal tissue*	Positive samples (%)
Heart	0/3 (0%)
Brain	0/3 (0%)
Liver	0/3 (0%)
Kidney	0/3 (0%)
Spleen	0/3 (0%)
Adrenal Gland	0/1 (0%)
Lung	0/3 (0%)
Skin	0/1 (0%)
Skeletal Muscle	0/3 (0%)
Thyroid	1/3 (33%)
Testis	3/3 (100%)

(\* ) Normal tissues from non-cancerous patients

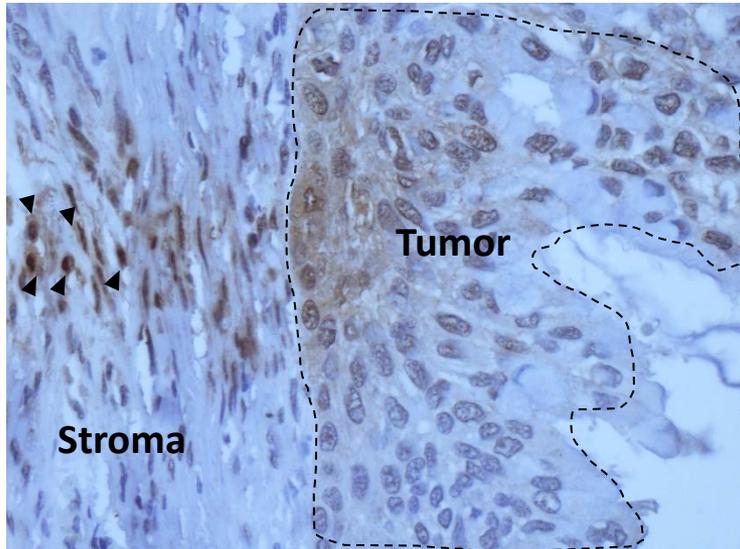


Lung tumor	Positive samples (%)
Adenocarcinoma	10/21 (48%)
Squamous carcinoma	3/12 (25%)
Undifferentiated carcinoma	2/4 (50%)
Bronchioalveolar, other	1/2 (50%)
<b>Total</b>	<b>16/39 (41%)</b>



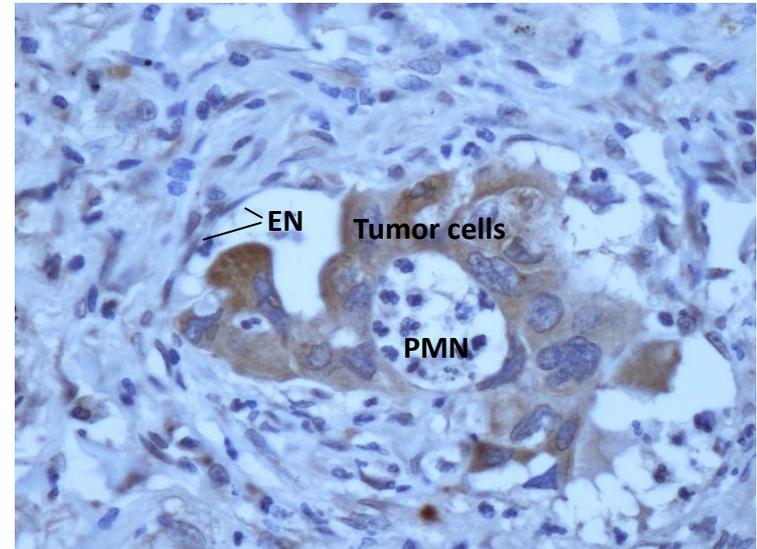
# Immunohistochemistry Analysis of Brachyury Expression in Lung Tumor Tissues

**Adenocarcinoma**



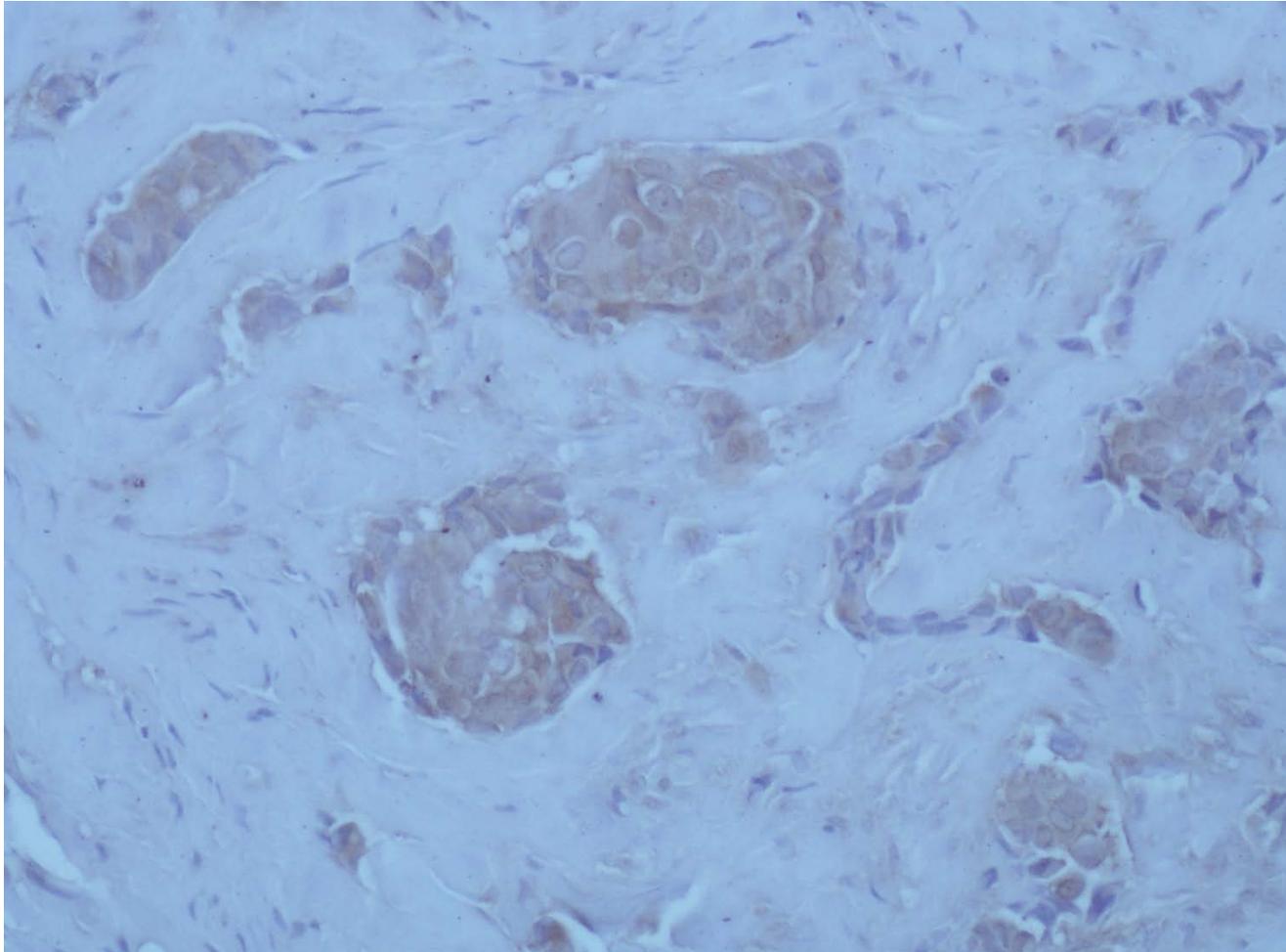
**Brachyury expression in tumor cells as well as in individual cells within the stroma of the tumor (indicated by arrowheads)**

**Adenocarcinoma**



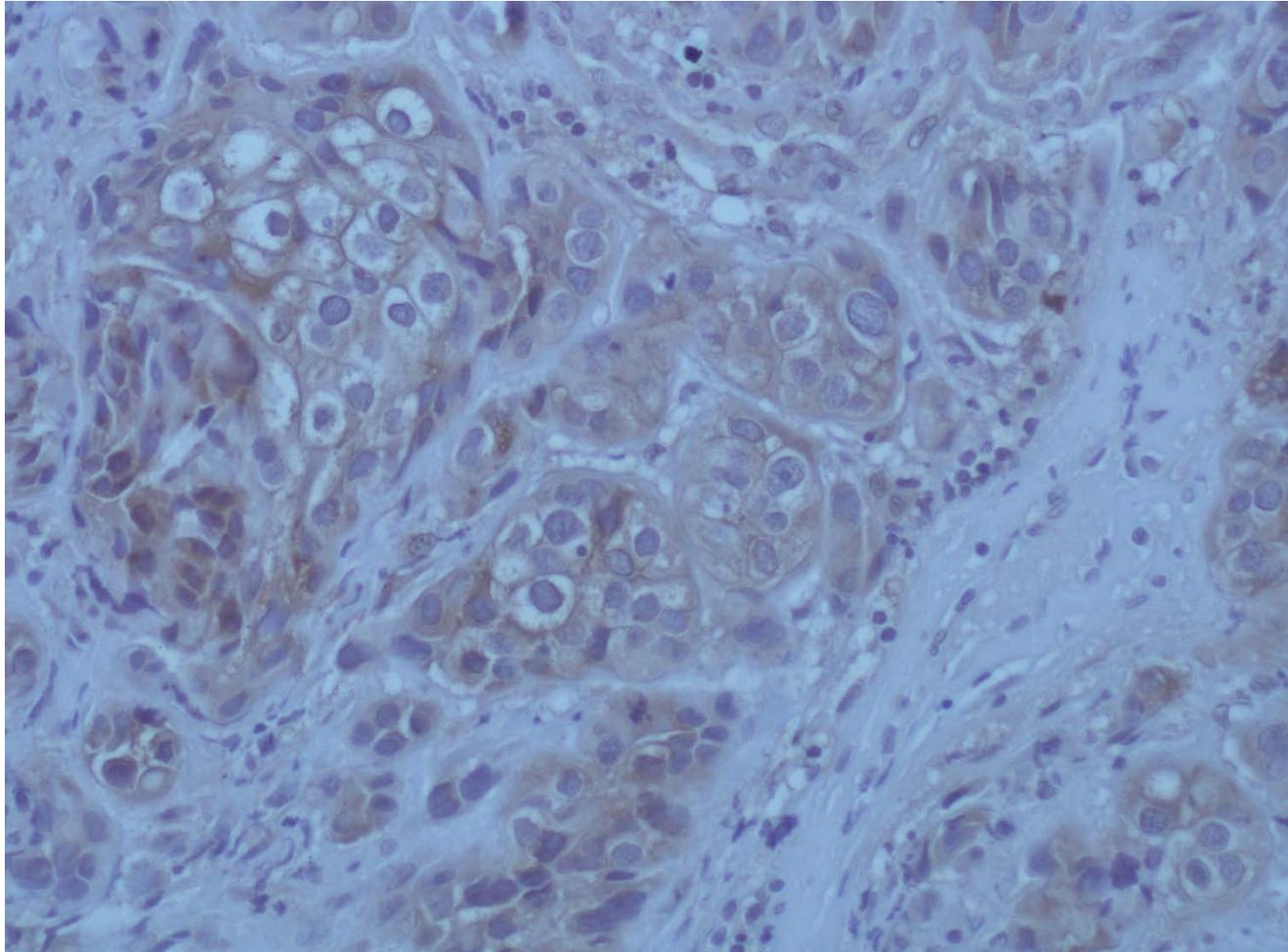
**Brachyury expression in tumor cells invading a blood vessel (EN: endothelial cells; PMN: polymorphonuclear leukocytes)**

# Immunohistochemistry Analysis of Brachyury Expression in a Bone Metastatic Lesion of a Breast Cancer Patient

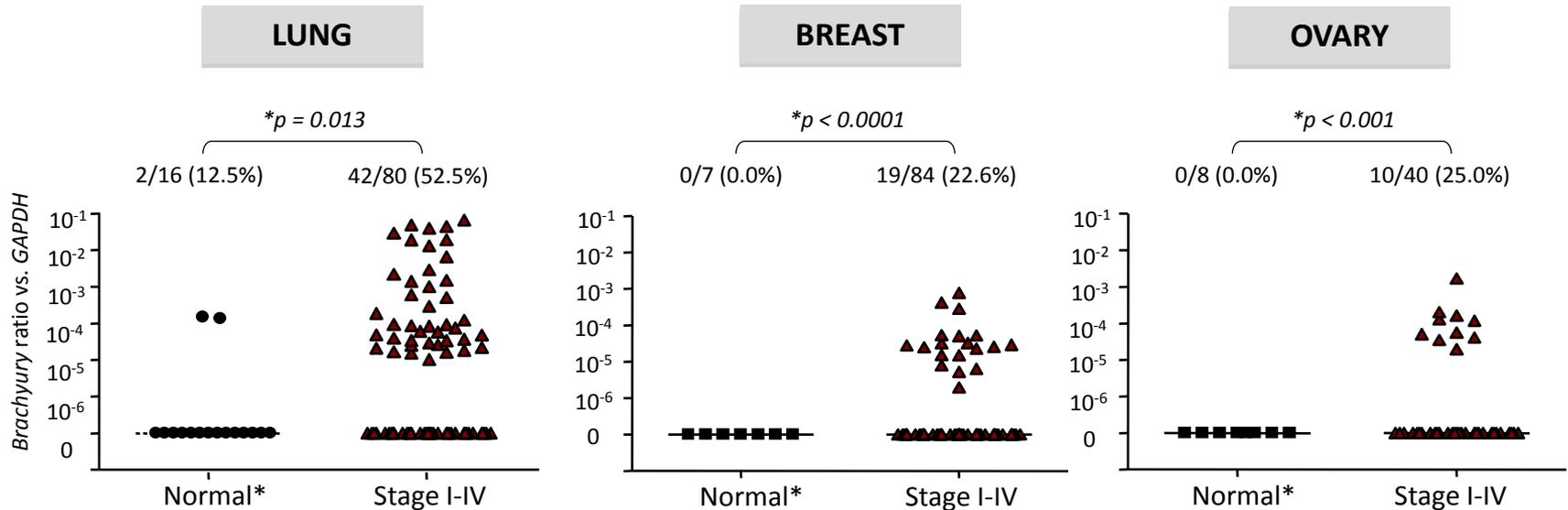


Case #2

# Immunohistochemistry Analysis of Brachyury Expression in a Brain Metastatic Lesion of a Breast Cancer Patient



# Brachyury Expression in Human Tumors and Normal Tissues



\* Normal tissue corresponds to a histologically normal area adjacent to the tumor.

# Expression of Brachyury and other tumor-associated antigens in human normal tissues

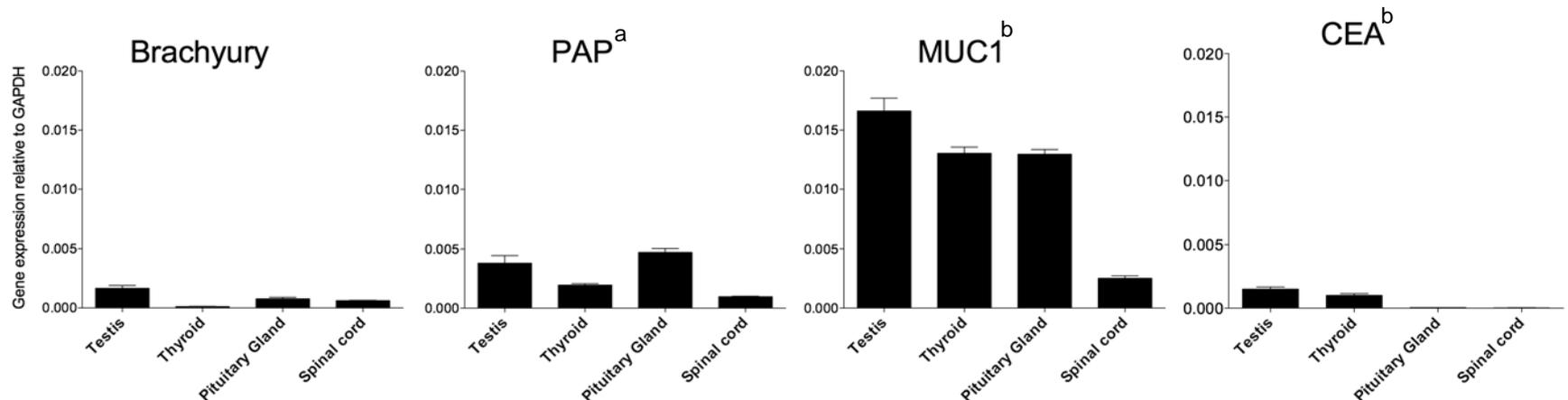
**Expression of Brachyury was *NEGATIVE* in the following human normal tissues:**

Heart, Lung, Esophagus, Stomach, Liver, Pancreas, Small Intestine, Colon, Kidney, Bladder, Ureter, Prostate, Ovary, Fallopian tube, Uterus (cervix), Placenta, Breast, Blood vessel (artery), Brain, Parathyroid, Adrenal, Spleen, Thymus, Bone Marrow, Lymph Node, Tonsil, Peripheral Leukocytes, CD34+ cells, Mononuclear cells, T cells (resting and activated), B cells (activated\*), Skeletal Muscle, Skin.

\*Resting B cells showed low levels of expression

**The following tissues showed some levels of Brachyury expression: comparison with other TAAs**

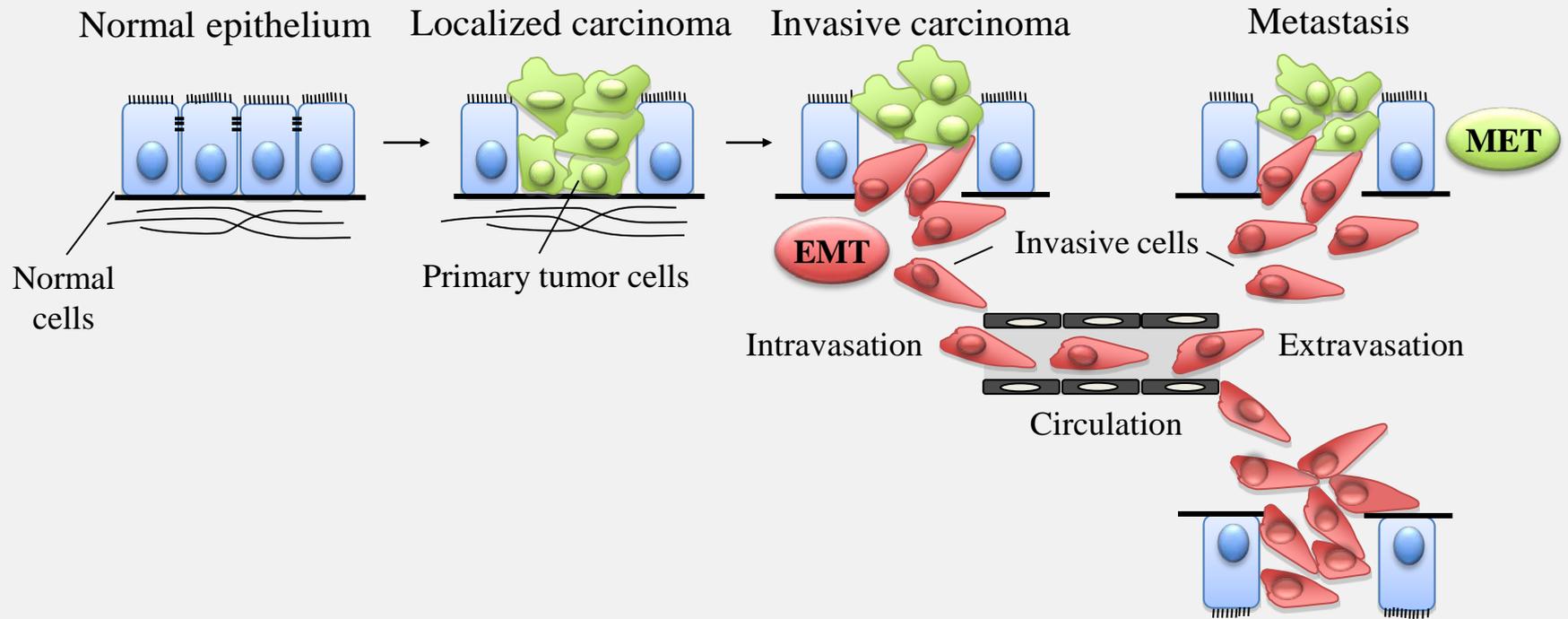
Testis, Thyroid, Pituitary Gland, Spinal cord



(a) Antigen in FDA approved Sipuleucel-T vaccine

(b) Antigens in multiple carcinoma targeting vaccines

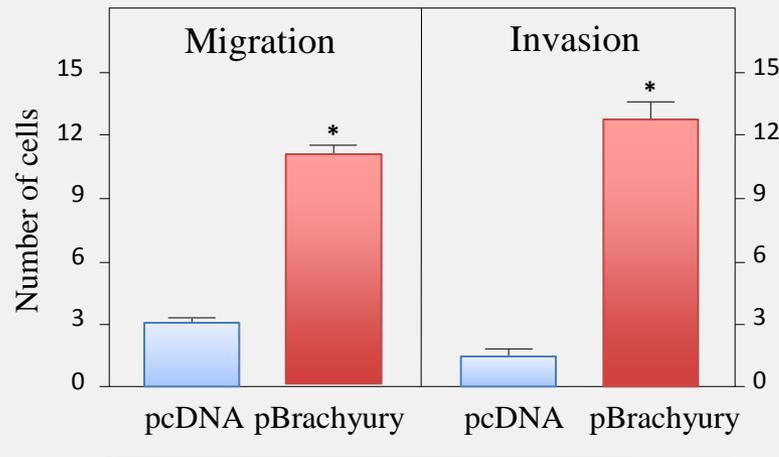
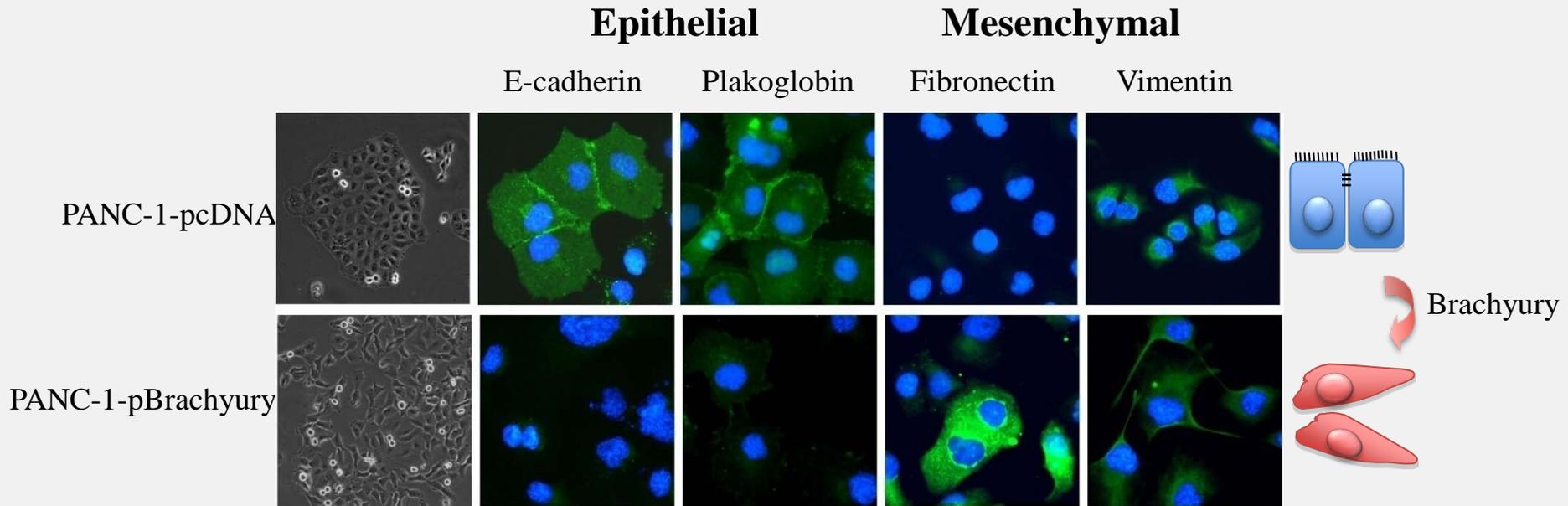
# Epithelial-to-Mesenchymal Transition (EMT): an Opportunity for Interventions Against Tumor Progression



## HYPOTHESIS

Vaccine strategies targeting essential regulators of the EMT process may be able to interfere with metastatic disease

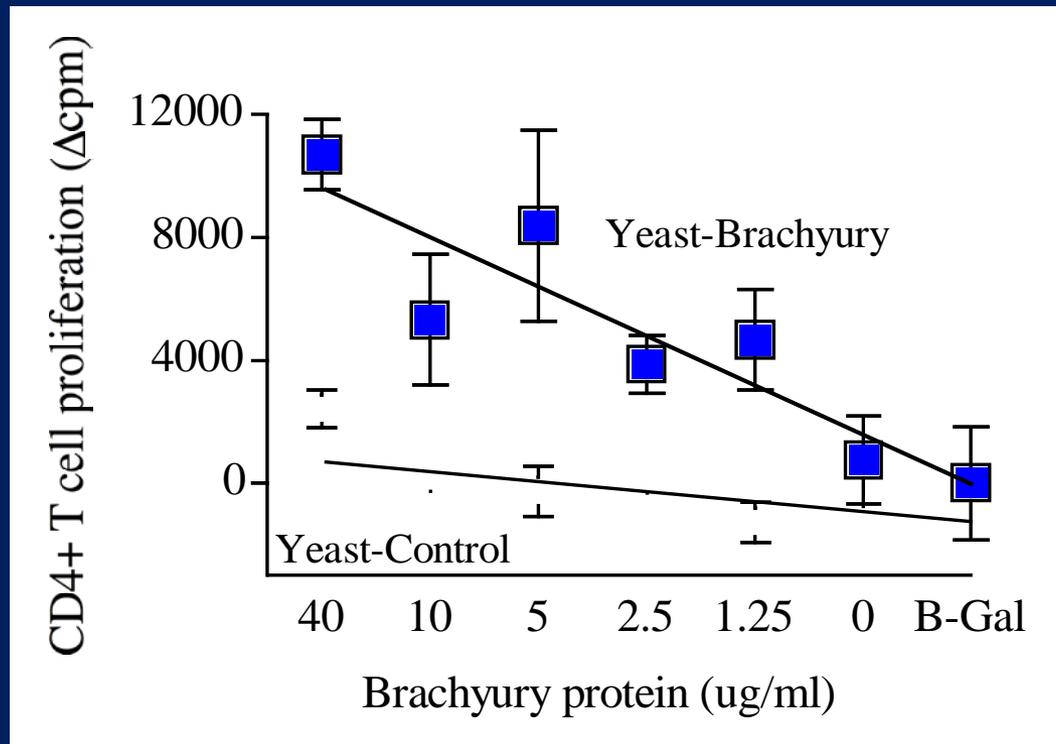
# Brachyury Overexpression Induces EMT in Epithelial Tumor Cells



PANC-1



# Ability of Yeast-Brachyury to Induce Brachyury-specific CD4<sup>+</sup> T-cell Responses

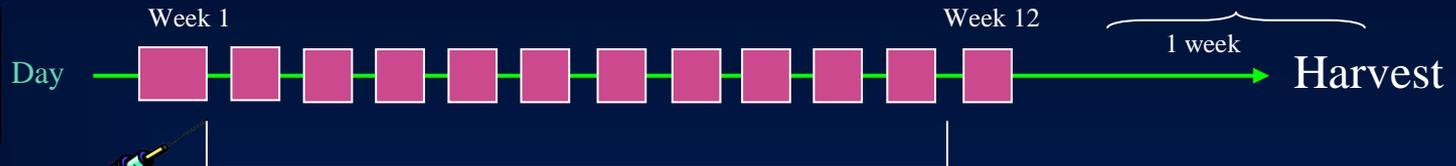


Mice were vaccinated at 4 weekly intervals with yeast-Brachyury or control yeast. Proliferation assays were carried out with purified Brachyury protein (from insect cells) and  $\beta$ -gal protein as control.

# Does Vaccination with Yeast-Brachyury Have Any Detrimental Effects in a Murine Model?



n=5/group  
Age Matched



Yeast-Brachyury (hu)

or

Yeast-WT

1 YU/site, 4 sites

or

No Vaccine

1 Vaccination/week,  
12 weeks  
(12 Vaccinations)

Analyses were done in the ‘spirit of GLP’ in a blinded fashion by DVM, MPH, DACVP, Charles River/Pathology Associates.

*There were no differences between groups in any measured parameters.*

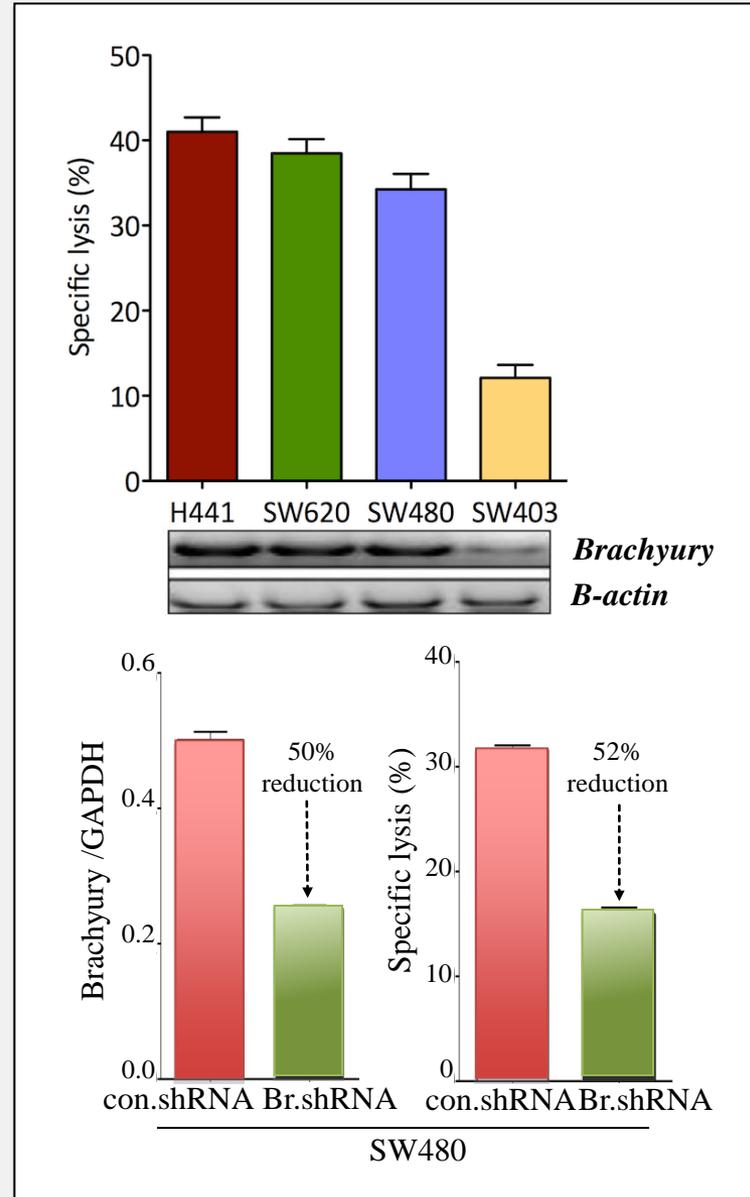
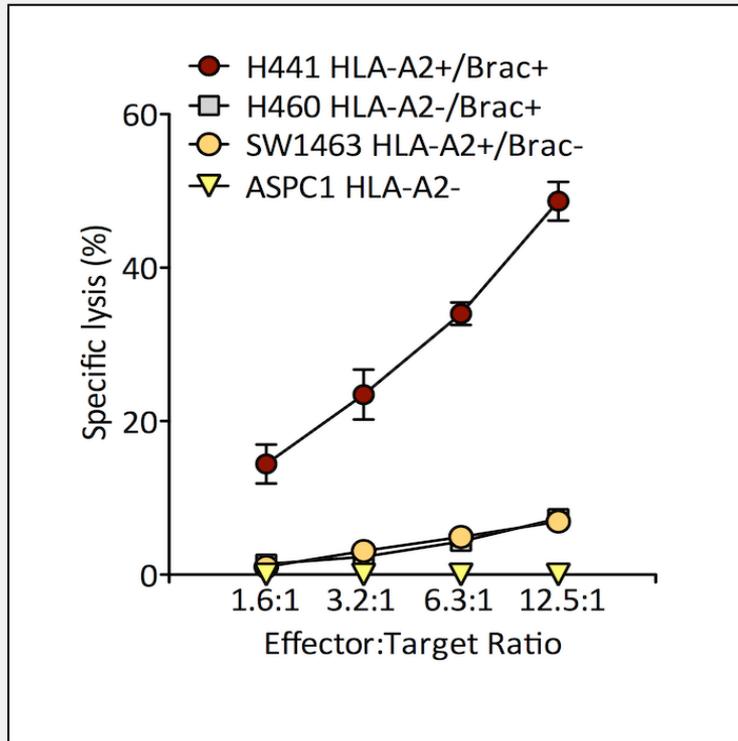
“Based on the findings of this study, vaccination with yeast-WT or yeast-human brachyury had no toxicity effects in normal mice.”

## Parameters:

- In-Life Body weight: 13 weeks
- Histopathology: 10 Tissues
- Serum Chemistry: 7 parameters
- CBC: 20 parameters
- Autoimmune Panel: 5 parameters

**Is Brachyury  
a Suitable Target for  
T-cell-Mediated  
Immunotherapy?**

# Brachyury-Specific T Cells Efficiently Lyse Tumor Cells That Are Brachyury Positive



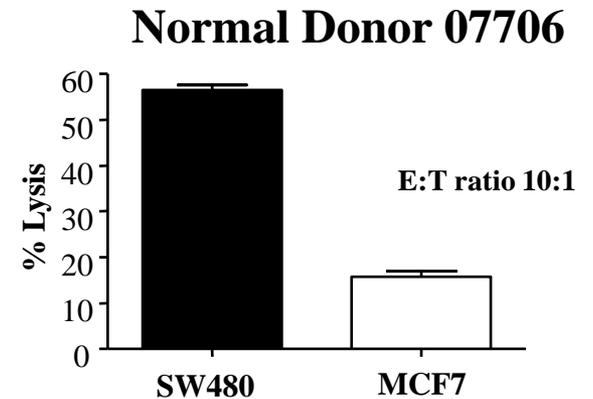
# CTL Lysis Mediated by Brachyury-Specific T Cells

CD8 cells isolated at IVS 3. CTL assay performed.

Overnight assay using HLA-A2+ / Brachyury<sup>high</sup>: SW480 cells

HLA-A2+ / Brachyury<sup>low</sup>: MCF7 cells

Normal Donor	Stimulation with:	Control Tetramer	Brachyury Tetramer
07706	Brachyury Yeast /Tp2	0.33	1.84
17663	Brachyury Yeast /Tp2	0.11	0.65
26532	Brachyury Yeast /Tp2	0.05	0.11



# Patients who have generated T-Cell Responses to Brachyury Post Vaccination

Pt	Vaccine	Metastatic Tumor Type	ELISPOT			
				PSA/CEA	Brachyury	HIV
1	PSA-TRICOM + $\alpha$ CTLA-4	Prostate Ca.	Pre Post	<1/200,000 1/150,000		<1/200,000 <1/200,000
2	PSA-TRICOM + $\alpha$ CTLA-4	Prostate Ca.	Pre Post	<1/200,000 1/40,000		<1/200,000 <1/200,000
3	Yeast-CEA	Medullary Thyroid Ca.	Pre Post	<1/200,000 1/9,677		<1/200,000 <1/200,000
4	Yeast-CEA	Colorectal Ca.	Pre Post	<1/200,000 <1/200,000		<1/200,000 <1/200,000

(Antigen cascade following tumor destruction and cross-presentation)

- **No autoimmune thyroid events or thyroid function changes in patients 1, 2, and 4. Patient 3 had prior thyroidectomy.**
- **ANA remained negative for patients 1 and 2 and TSH within normal limits. No autoimmune work-up was performed or indicated for patients 3 and 4.**

# Brachyury as a Vaccine Target for the Control of the EMT Phenotype

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- High degree of selective expression of Brachyury in human tumors vs. normal adult tissues
- Human T cells can be generated in vitro using:
  - (a) a Brachyury-specific peptide
  - (b) yeast-Brachyury vaccine
- T cells generated
  - lyse human carcinoma cells endogenously expressing Brachyury
- Patients receiving PSA-TRICOM vaccine plus anti-CTLA-4 and yeast-CEA vaccine developed Brachyury T-cell responses post-vaccination

# Phase 1 Clinical Trial of GI-6301 (*S. cerevisiae*-brachyury)

- Open label, phase 1 trial
- Three sequential dose cohorts will be explored (n=3-6/group)
  - 4 YU SC, 16 YU, and 40 YU SC
  - Each total dose is split into 4 injections (4 YU= 1 YU x 4 injections)
- Dosing: days 1, 15, 29, 43, 57, 71, 85 then monthly until DLT or PD
- Decision to escalate based on DLTs
  - If 0 of 3 subjects develop DLT, then the dose may be escalated
  - If 1 of 3 subjects develops DLT, then up to 3 more subjects will be enrolled
  - If no more than 1 of 6 subjects develops DLT, then the dose will be escalated
  - If 2 or more subjects experience DLT, then the MTD has been exceeded
- Careful clinical evaluation with attention to thyroid and pituitary dysfunction will be carried out in collaboration with endocrinology from NIDDK (Dr. Celi)

# Phase 1 Clinical Trial of GI-6301 (*S. cerevisiae*-brachyury)

- Expansion of MTD dose group by 10 additional subjects is planned
- Immune analysis to include
  - ELISPOT for brachyury and cascade antigens (CEA, MUC-1 etc.)
  - Immune cell phenotype and function (CD4, CD8, Treg, MDSC etc.)

# Study Status

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1. Pre-IND meeting with FDA 8 July 2011
2. NCI Scientific Review approved 29 July 2011
3. NCI IRB approved (provisional) 22 Aug 2011
4. NCI IBC approved 07 Sept 2011
5. RAC meeting 13 Sept 2011

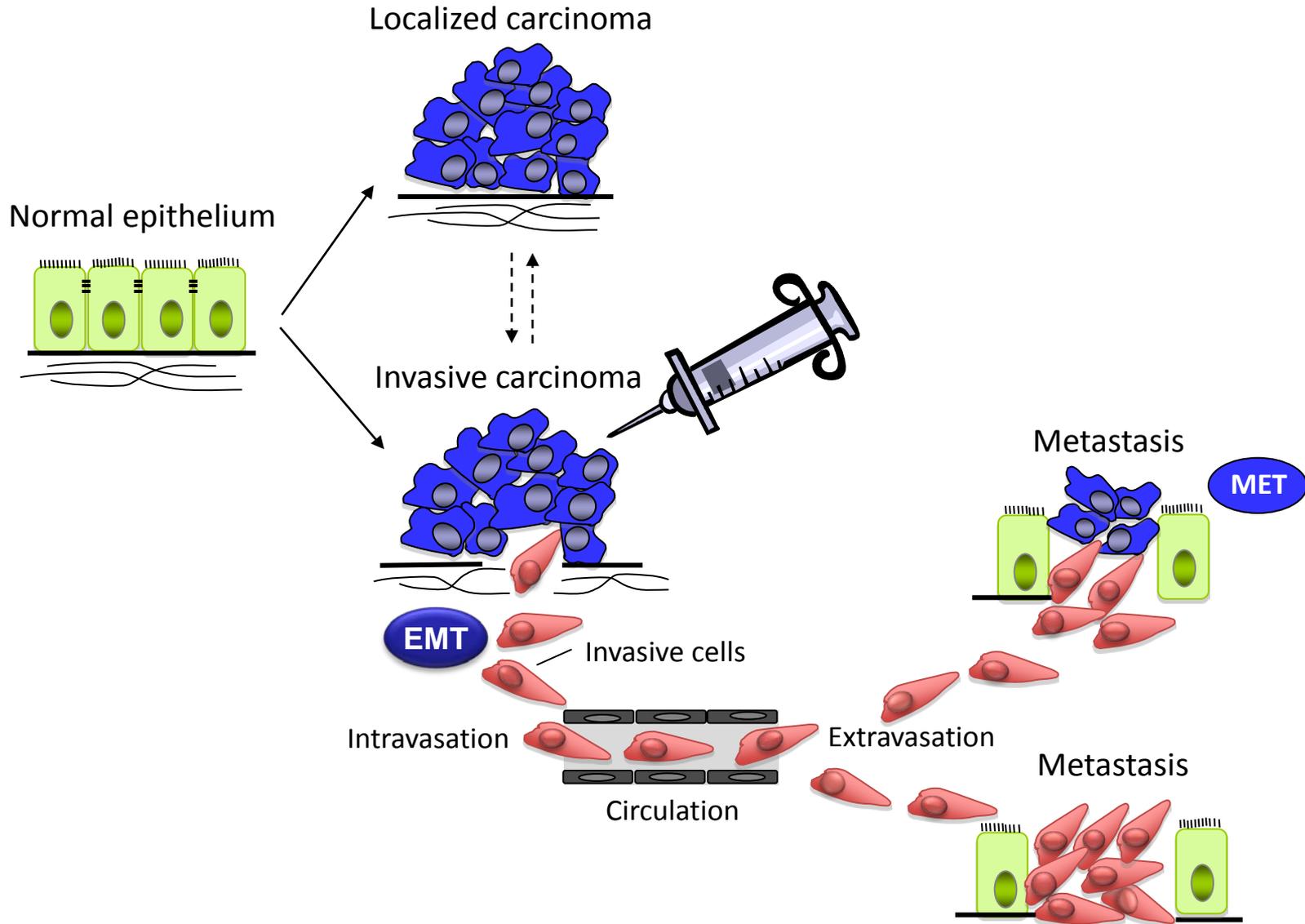
# Comments from RAC

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**NF1 and MS1:** Why not limit enrollment to subjects with tumors expressing Brachyury

1. Plasticity of EMT
2. Higher proportion of brachyury expressing cells in metastasis than primary
  - Primary tumor usually available. Tissue from metastatic lesion not usually available.
  - Biopsies of primary may give false negative (miss the few brachyury expressing cells at tumor margin)
3. Primary endpoints are safety and immunogenicity (co-primary endpoint for expansion).

# EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT): ROLE IN TUMOR PROGRESSION

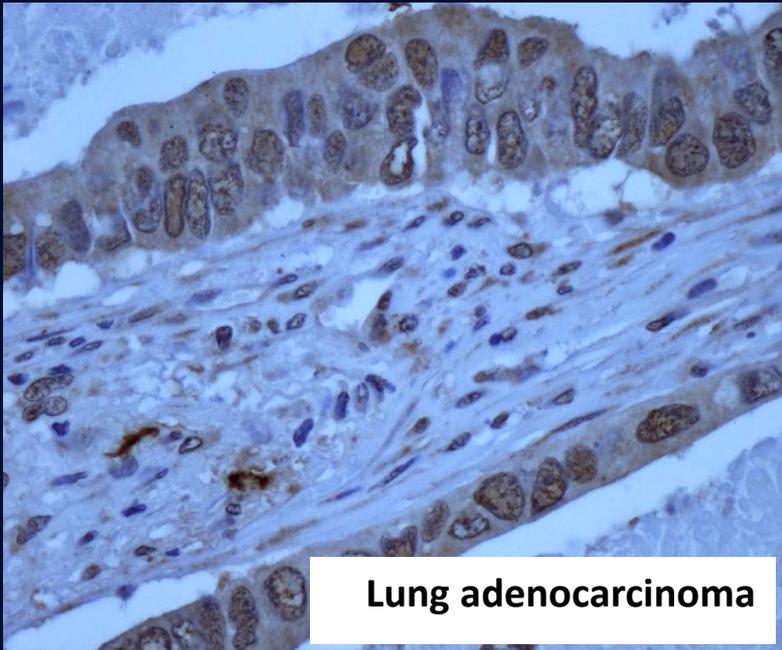


# Comments from RAC

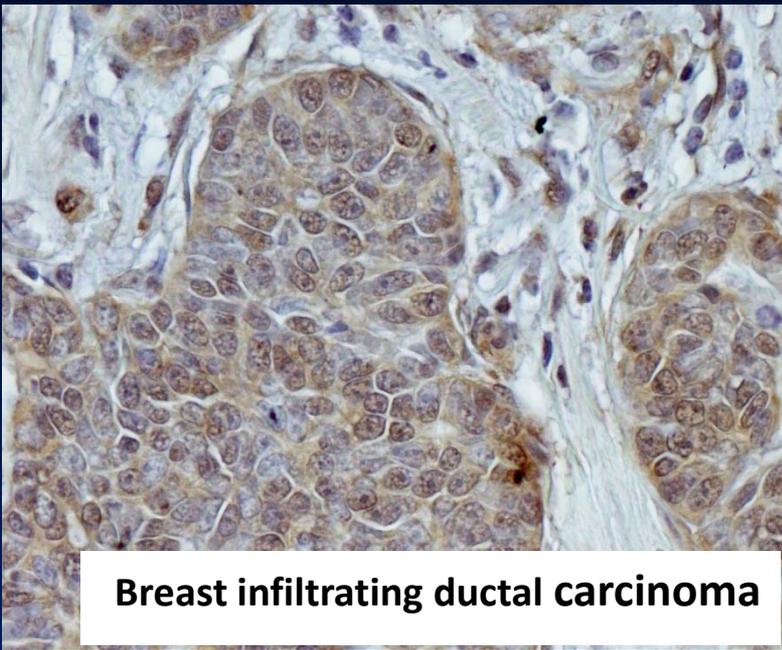
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## **MSK1 and 2:** Brachyury on B-cells

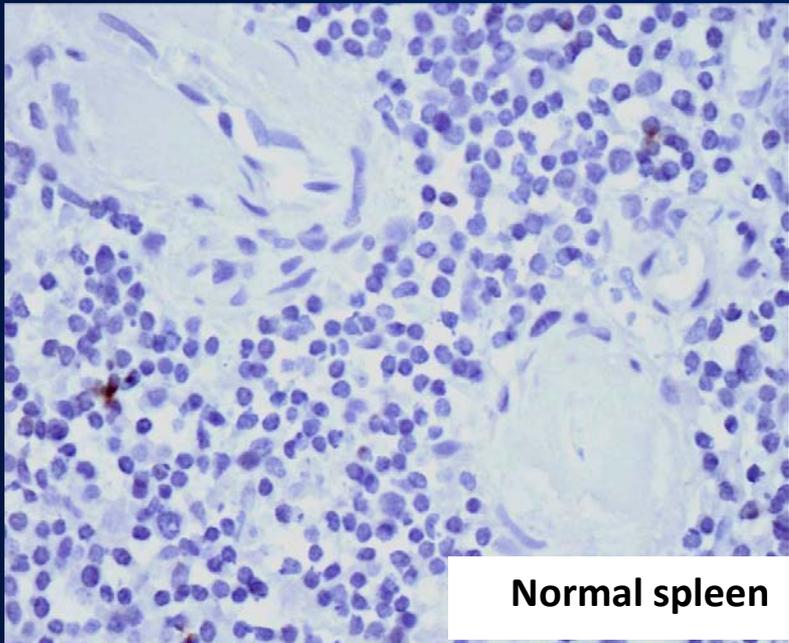
1. Brachyury not seen by IHC in spleen and lymph nodes which both contain high levels of B-cells
2. Normal B-cells not lysed by brachyury specific T-cells
3. Only seen by PCR in pooled samples of B-cells.
  - Appears to be related to EBV expression
    - EBV infection of B-cells induces a large increase in Brachyury
  - ~95% of adults previously infected with EBV → latent infection
  - EBV expression is on average between 1 in  $10^5$  and 1 in  $10^6$  PBMCs.
    - PCR only positive at high number of cycles



**Lung adenocarcinoma**

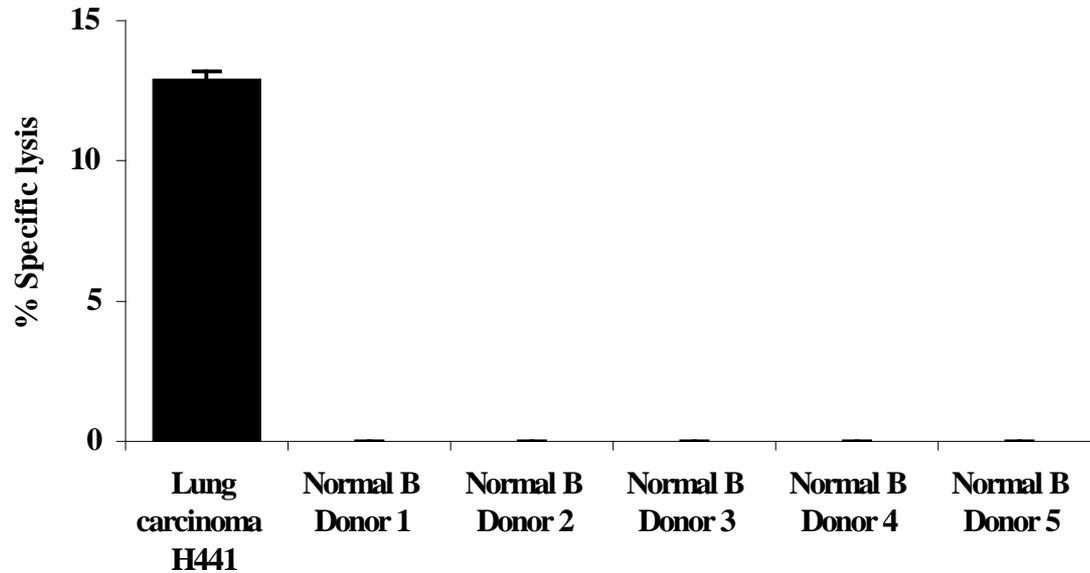


**Breast infiltrating ductal carcinoma**



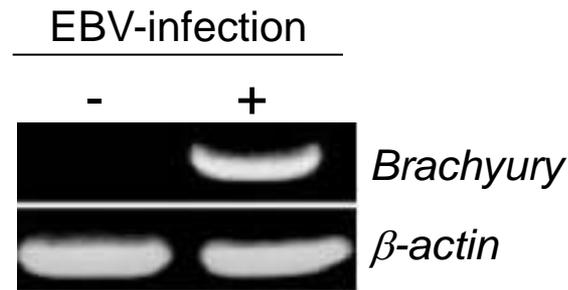
**Normal spleen**

# Brachyury-specific T cells do not lyse normal B lymphocytes



Brachyury-specific T cells were generated in vitro from the blood of a cancer patient by stimulation with a Brachyury 9-mer peptide that binds to the HLA-A2 molecule. Lung carcinoma H441 cells (HLA-A2+) were used as a positive control. B cells (HLA-A2+) were isolated from peripheral blood (CD19+ fraction) of 5 normal donors and evaluated as targets in a 5-hour <sup>111</sup>In release assay.

# Infection of human normal B lymphocytes with EBV virus enhances the expression of Brachyury



B cells from peripheral blood of a healthy donor were left uninfected (-) or were infected with EBV (+). RNA was isolated and RT-PCR was performed for Brachyury and  $\beta$ -actin.

# Comments from RAC

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## **MSK1 and 2:** Brachyury on B-cells (safety)

1. The best explanation of the positive PCR and negative IHC and lysis is a small number of latent EBV infected B-cells in some patients. Any therapeutic brachyury specific immune response to these cells poses no increased risk.
2. We are obtaining CBC and flow cytometry for B-cells (CD-19) pre and post vaccination (D29 and D85)

# Comments from RAC

## MSK5: Research Immune Assays and time points

### Section 6.3 and Appendix D

Assay	Primary analysis (pre and day 85)	Other samples (each visit)
ELISPOT	+	+/-
NK	+	+/-
Treg	+	+/-
Humoral responses	+	+/-
Others	+/-	+/-

- Other sample time points allow for determination of kinetics of immune response and correlations with clinical outcomes (adverse events or responses)
- Also serves as additional samples for future immune assays
- Amount well within NCI IRB guidelines for limits of obtaining research blood samples

# Comments from RAC

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**MS2:** Why not limit selection to locally advanced regional pathology

1. This is a very forward thinking question. Eventually that is exactly where we want to go.
  - Metastatic lesions greatly over express brachyury so it remains a very reasonable target for a therapeutic vaccine
  - For this first-in-human study, after discussion with our scientific review committee, our IRB our IBC and the FDA we feel that the most appropriate initial safety study would be in patients with metastatic disease.