CD22 Antigen Is Broadly Expressed on Lung Cancer Cells and Is a Target for Antibody-Based Therapy

Joseph M. Tuscano, Jason Kato, David Pearson, Chengyi Xiong, Laura Newell, Yunpeng Ma, David R. Gandara, and Robert T. O'Donnell

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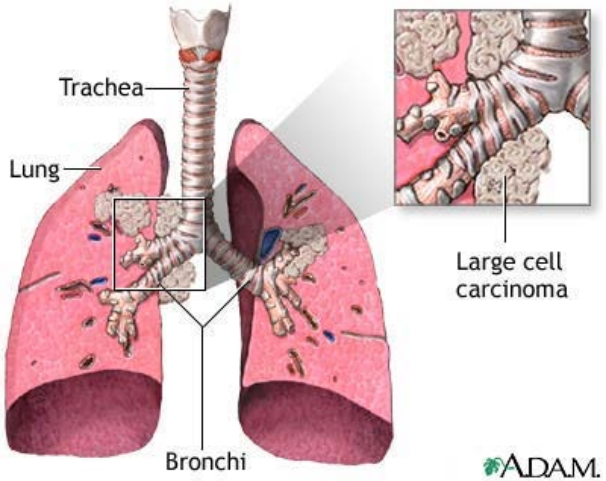
Labmeeting 12.11.2012

Lung Cancer

Worldwide in 2010, an estimated 1.7 million new cases of lung cancer were expected to be diagnosed, accounting for approximately 13 percent of total cancer diagnoses. Lung cancer is the leading cause of cancer death worldwide, with an estimated 1.4 million deaths each year.



In Europe the third most common cancer with an incidence of 60 per 100 000, after breast/prostate and colon/rectum cancer
The

The International Agency for Research on Cancer, the World Health Organization, GLOBOCAN 2008.

Leading New Cancer Cases and Deaths – 2012 Estimates

Estimated New Cases*

Male		Female	
Prostate 241,740 (29%)		Breast 226,870 (29%)	
Lung & bronchus 116,470 (14%)	14%	Lung & bronchus 109,690 (14%)	14%
Colon & rectum 73,420 (9%)		Colon & rectum 70,040 (9%)	
Urinary bladder 55,600 (7%)		Uterine corpus 47,130 (6%)	
Melanoma of the skin 44,250 (5%)		Thyroid 43,210 (5%)	
Kidney & renal pelvis 40,250 (5%)		Melanoma of the skin 32,000 (4%)	
Non-Hodgkin lymphoma 38,160 (4%)		Non-Hodgkin lymphoma 31,970 (4%)	
Oral cavity & pharynx 28,540 (3%)		Kidney & renal pelvis 24,520 (3%)	
Leukemia 26,830 (3%)		Ovary 22,280 (3%)	
Pancreas 22,090 (3%)		Pancreas 21,830 (3%)	
All sites 848,170 (100%)		All sites 790,740 (100%)	

Estimated Deaths

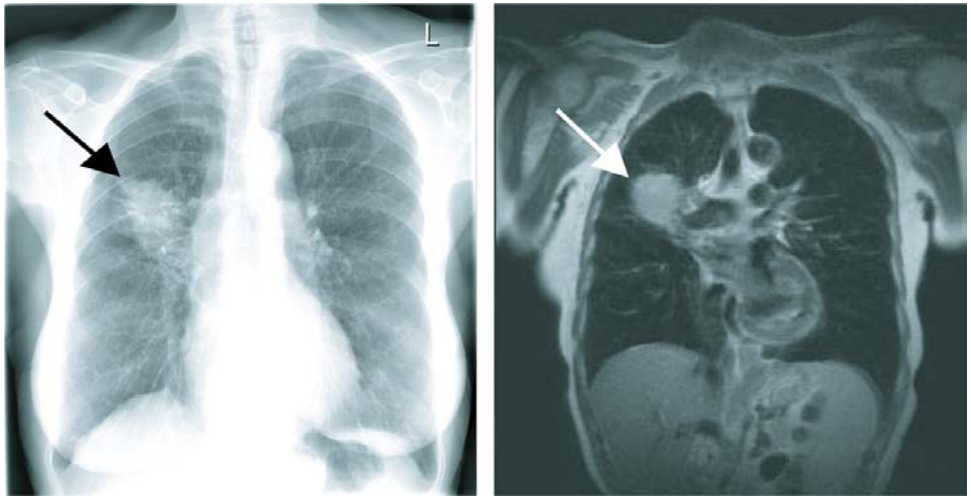
Male		Female	
Lung & bronchus 87,750 (29%)	29%	Lung & bronchus 72,590 (26%)	26%
Prostate 28,170 (9%)		Breast 39,510 (14%)	
Colon & rectum 26,470 (9%)		Colon & rectum 25,220 (9%)	
Pancreas 18,850 (6%)		Pancreas 18,540 (7%)	
Liver & intrahepatic bile duct 13,980 (5%)		Ovary 15,500 (6%)	
Leukemia 13,500 (4%)		Leukemia 10,040 (4%)	
Esophagus 12,040 (4%)		Non-Hodgkin lymphoma 8,620 (3%)	
Urinary bladder 10,510 (3%)		Uterine corpus 8,010 (3%)	
Non-Hodgkin lymphoma 10,320 (3%)		Liver & intrahepatic bile duct 6,570 (2%)	
Kidney & renal pelvis 8,650 (3%)		Brain & other nervous system 5,980 (2%)	
All sites 301,820 (100%)		All sites 275,370 (100%)	

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Cancer Facts & Figures 2012. *American Cancer Society*

Prognosis in lung cancer according to clinical stage		
Clinical stage	Five-year survival (%)	
	Non-small cell lung carcinoma	Small cell lung carcinoma
IA	50	38
IB	47	21
IIA	36	38
IIB	26	18
IIIA	19	13
IIIB	7	9
IV	2	1

Rami-Porta, R; Crowley JJ, Goldstraw P (February 2009). "The revised TNM staging system for lung cancer". Annals of Thoracic and Cardiovascular Surgery 15 (1): 4–9.



Chest x-ray and MRI scan - NSCLC Adenocarcinoma (pT2 pN2, IIIA)

Risk Factors

Lung cancer risk factors include:

- Smoking cigarettes or cigars exposure to second-hand smoke, asbestos, radon, chromium, arsenic, soot or tar
- Treatment with radiation therapy to the breast or chest
- Personal or family history of the disease

Most lung cancers do not cause any symptoms until the disease has already reached an advanced stage. Even when symptoms do appear, they can be mistaken for other health problems.

American Cancer Society. Detailed Guide: Lung Cancer (Non-Small Cell). Available at: http://www.cancer.org/acs/groups/cid/documents/webcontent/003115-pdf.pdf. Accessed June 26, 2012.

Picture: Rüttinger et al. Journal of Translational Medicine 2007 5:43

Lung Cancer: Classification and Treatment

Classified into two major types:
non-small cell lung cancer (NSCLC) – 85%
small cell lung cancer (SCLC)

NSCLC Treatment

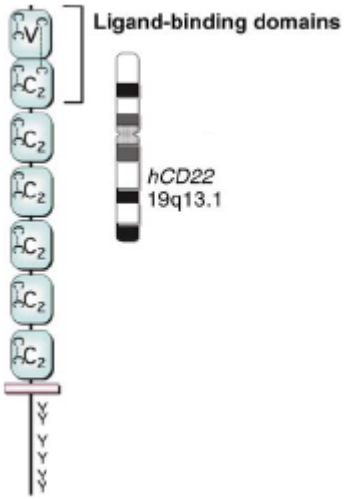
Despite treatment, the outlook for patients with NSCLC, particularly in the metastatic or locally advanced setting (Stage IIIB/IV), is generally poor.

Current treatment options for NSCLC include surgery, radiation therapy, chemotherapy, targeted therapy, or some combination of these, depending on the type of cancer, stage of the disease and overall health and age of the patient

The current first line standard of care with chemotherapy-based regimens for advanced NSCLC demonstrates a response rate of about 24-30 percent, with a median survival of 10.3 to 12.3 months and a progression free survival (PFS) of 4.8 to 5.1 months.23

American Cancer Society. Detailed Guide: Lung Cancer (Non-Small Cell). Available at: http://www.cancer.org/acs/groups/cid/documents/webcontent/003115-pdf.pdf. Accessed June 26, 2012.

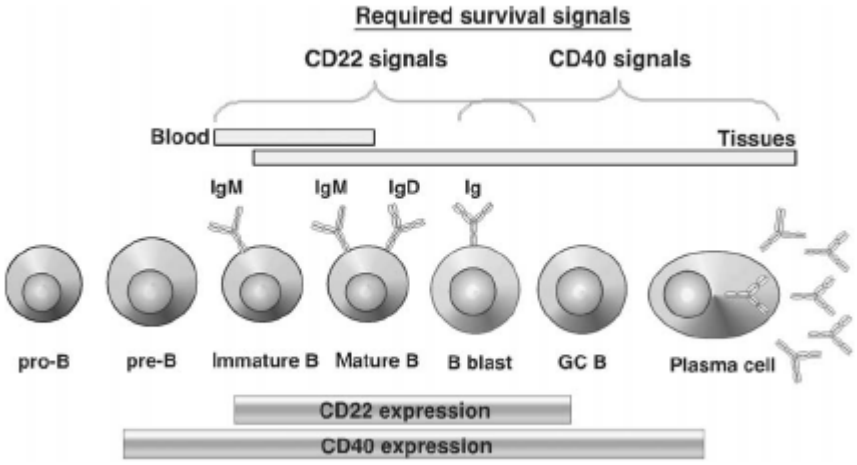
CD 22



B lymphocytes are the central mediators of humoral immunity. Th differentiate through highly regulated pathways before becoming mature plasma cells that secrete antigen (Ag)-specific antibody

The functions of B-cells primarily rely on signals generated by the B-cell Ag receptor (**BCR**)

CD22 and CD19 represent two specialized costimulatory or coreceptor cell surface molecules
‘‘response regulators’’



Tedder TF, Poe JC, Haas KM. CD22: a multifunctional receptor that regulates B lymphocyte survival and signal transduction. Adv Immunol 2005;88:1–50.

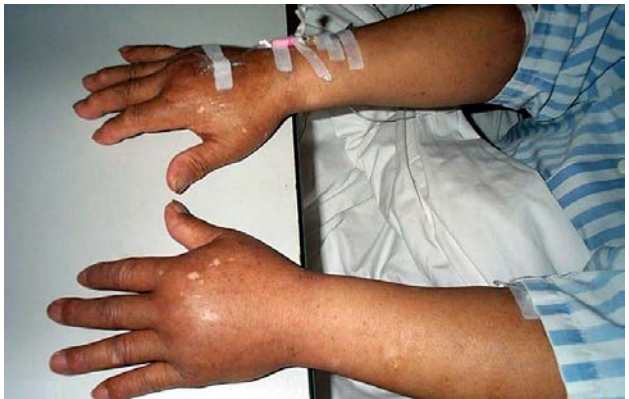
CD22 plays an important role in regulating mature B-cell activation, effector function, and survival in vivo, but its loss does not have dramatic effects on early B-cell development, maturation or differentiation.

CD22 is expressed by 60–80% of B-cell lymphomas and leukemias

In vitro CD22 mAb mediates antibody- and complement-dependent cytotoxicity, providing a rationale for its use in immunotherapy

CD22 mAb-immunotoxins have been evaluated in phase I–II clinical trials, with partial or transient clinical remissions

But severe and in certain cases fatal vascular leak syndrome was dose limiting



Recent clinical trial using a recombinant CD22-Pseudomonas exotoxin-based immunotoxin induced complete remissions in 68% of chemorefractory hairy-cell leukemia patients

Tedder TF, Poe JC, Haas KM. CD22: a multifunctional receptor that regulates B lymphocyte survival and signal transduction. Adv Immunol 2005;88:1–50.

Methods

the recent finding of expression CD22 in lung cancer make it a possible target for anti-CD22 mAb therapie

Flow cytometry

Real-time PCR

Western blot analysis

Northern blot analysis

Immunohistochemistry

In vitro cytotoxicity

Xenograft studies

I-PET

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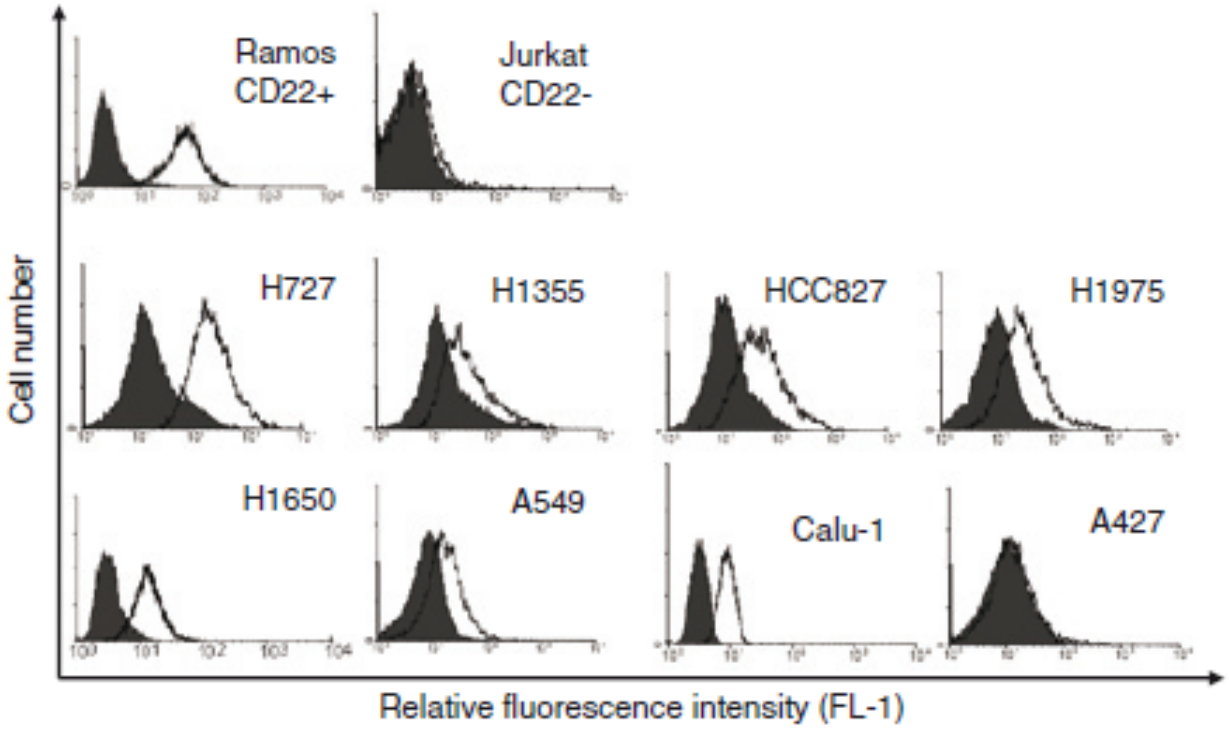
Results: Expression of CD22 in lung cancer



FACS Expression of CD22 in lung cancer. Ramos and Jurkat cells served as CD22-positive and negative controls

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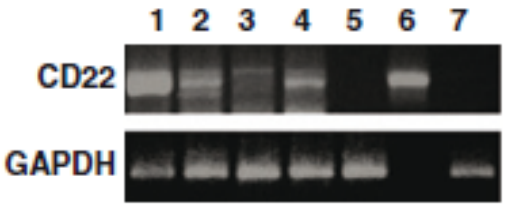
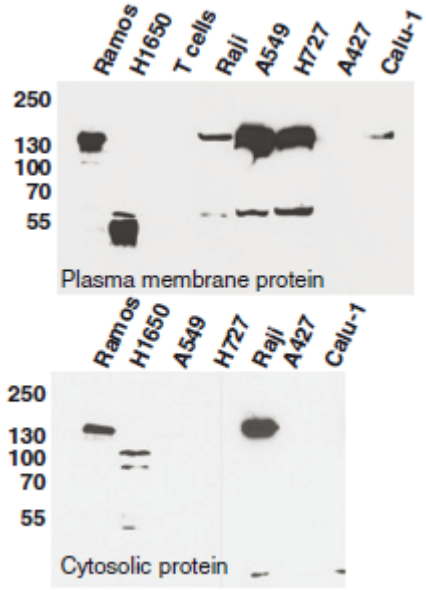


PCR amplification of CD22 from designated cDNA (lane 1, Ramos; lane 2, A549; lane 3, H1650; lane 4, H727; lane 5, A427; lane 6, CD22 plasmid; lane 7, BEC)



Western blotting of CD22

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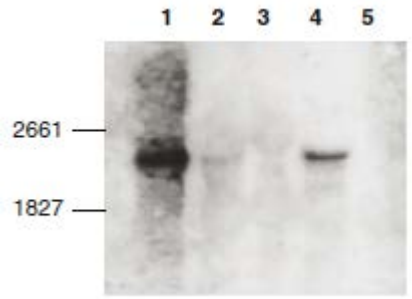
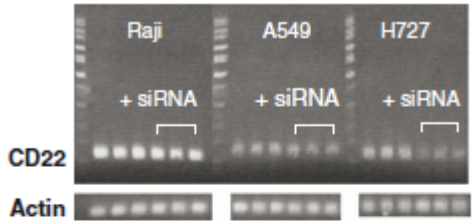
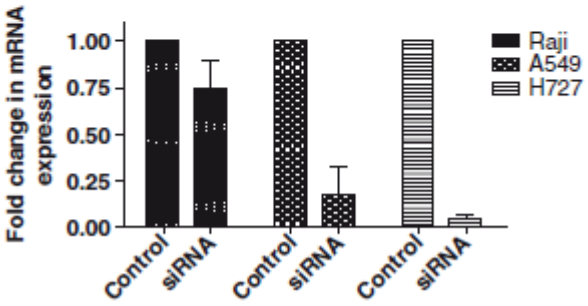


CD22 northern blot of total RNA extracted from Ramos, A549, H1650, H727, and A427 cells





To further validate CD22 gene expression, an siRNA approach coupled with quantitative real time PCR was used used. Transfection of Raji, A549, and H727 cells with CD22 siRNA resulted in a significant reduction in CD22 mRNA expression

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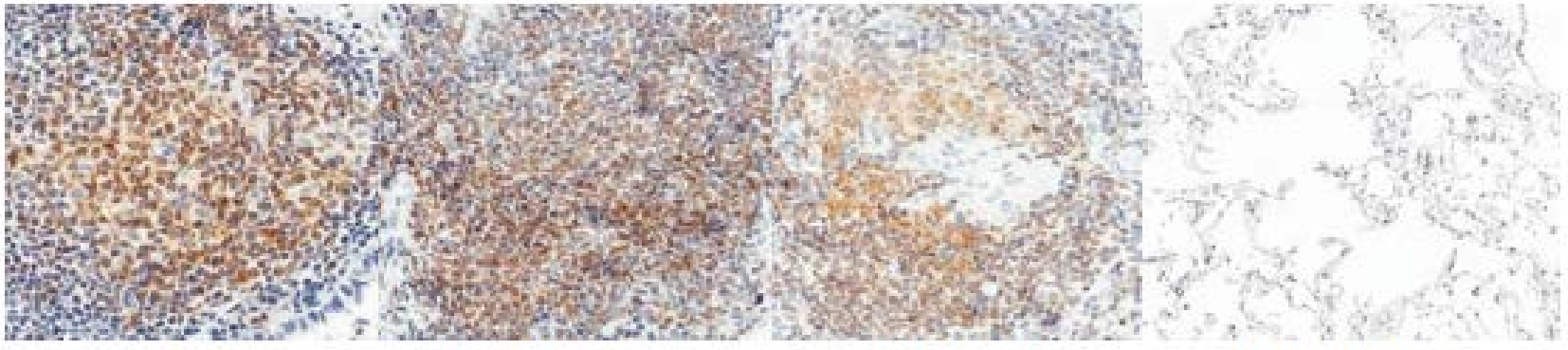

Immunohistochemistry: The anti-CD22 signal was often intense in part of the tumors, but weak or undetectable in the surrounding normal lung tissue. Several additional NSCLC patient specimens also stained CD22-positive

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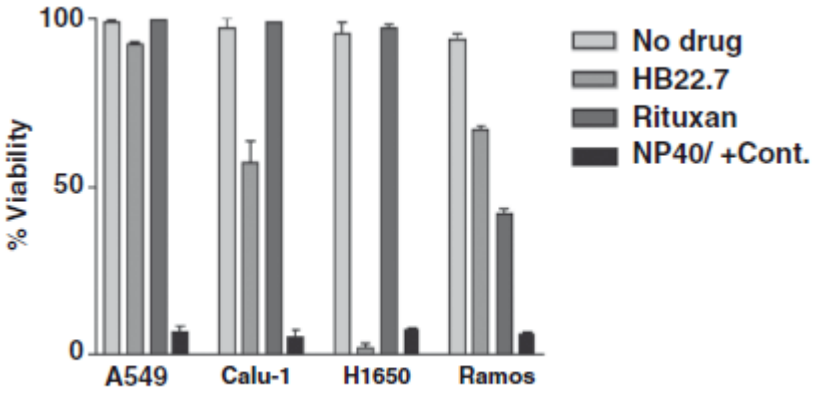
Results: CD22-mediated cytotoxicity



Three lung cancer cell lines, as well as Ramos cells, were tested for their responsiveness to HB22.7. After 72 hours The viability of H1650 and Calu-1 was reduced by 90% and 45%

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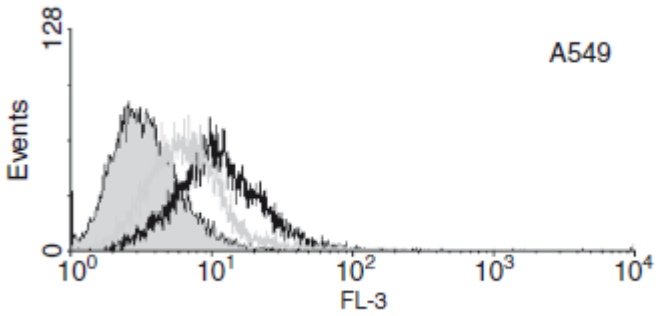
Results: Internalization of CD22

anti-CD22 mAbs mediate CD22 internalization in B cells and NHL . The degree of internalization is proportional to the degree of cytotoxicity. HB22.7 mediates more than 80% of CD22 internalization in NHL cells



Internalization of CD22 was also assessed by measuring surface-bound HB22.7. Anti-mouse IgG PE-Cy5 (secondary Ab) was used to detect HB22.7

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Results: Xenograft studies

whole body irradiation
(400 rads)

metastatic group received
5x10^6 tumor cells in tail vein

divided into groups

4d

84d /or when tumor
reached tumor reached 15mm

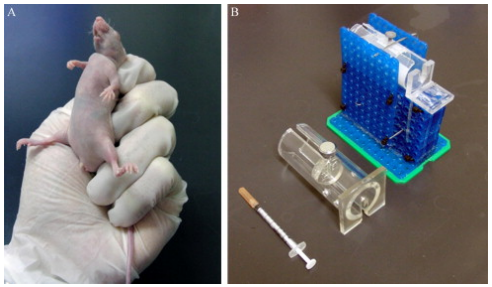
0d

3d

implantation of
5x10^6 tumor cells
subcutaneously on the
flank

Treatment
1.4 mg HB22.7

32d



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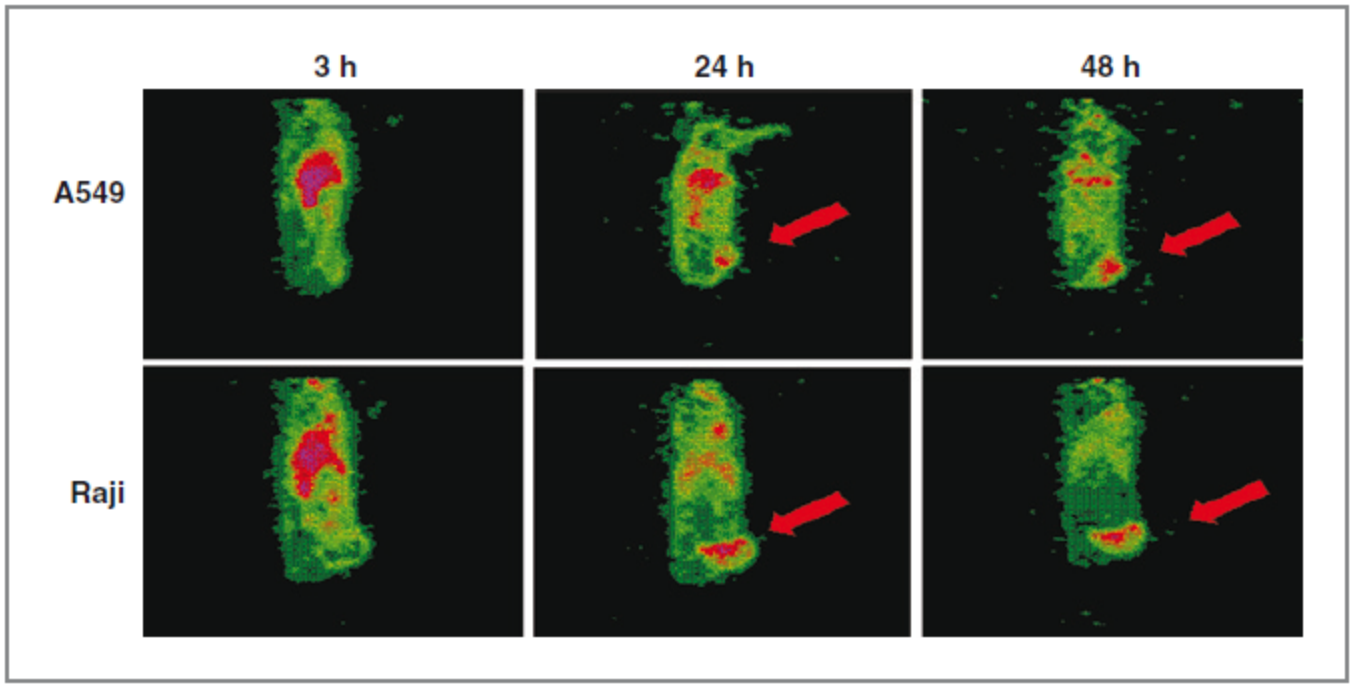
Results: : Xenograft studies – HB22.7 effectively targets NSCLC



Mice bearing A549 or Rajixenografts (arrow) received 64Cu-DOTA-HB22.7 (50 mCi) for I-PET using a micro-PET scanner.

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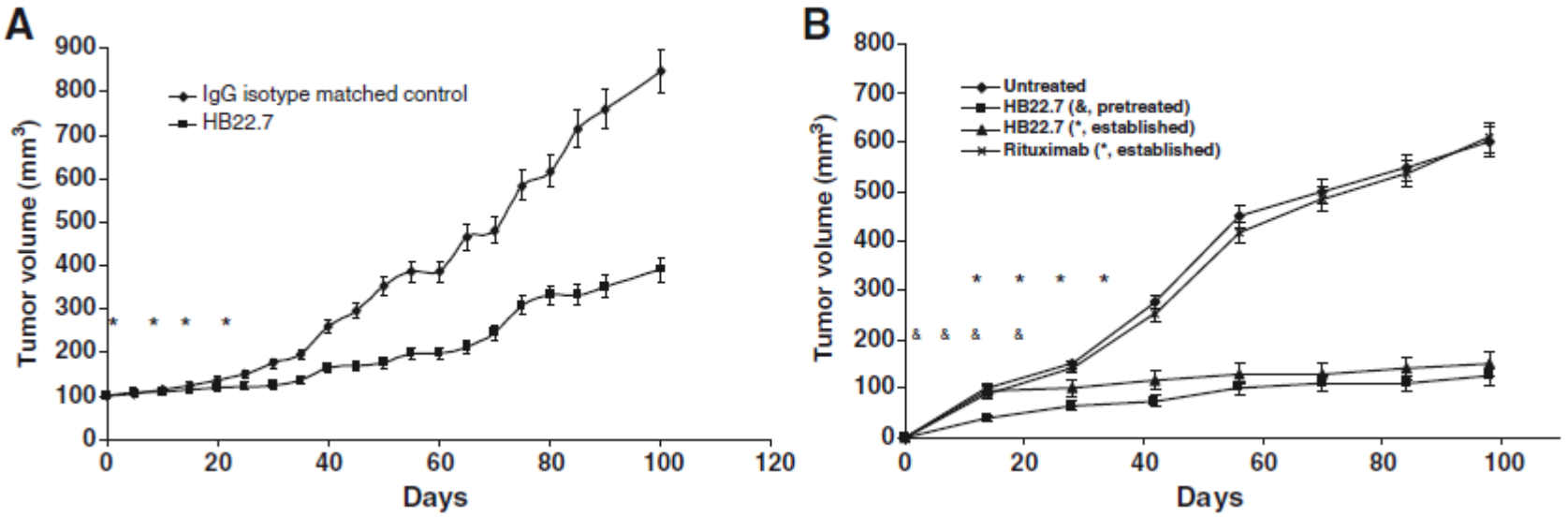
Results: : Xenograft studies – Tumor Volume



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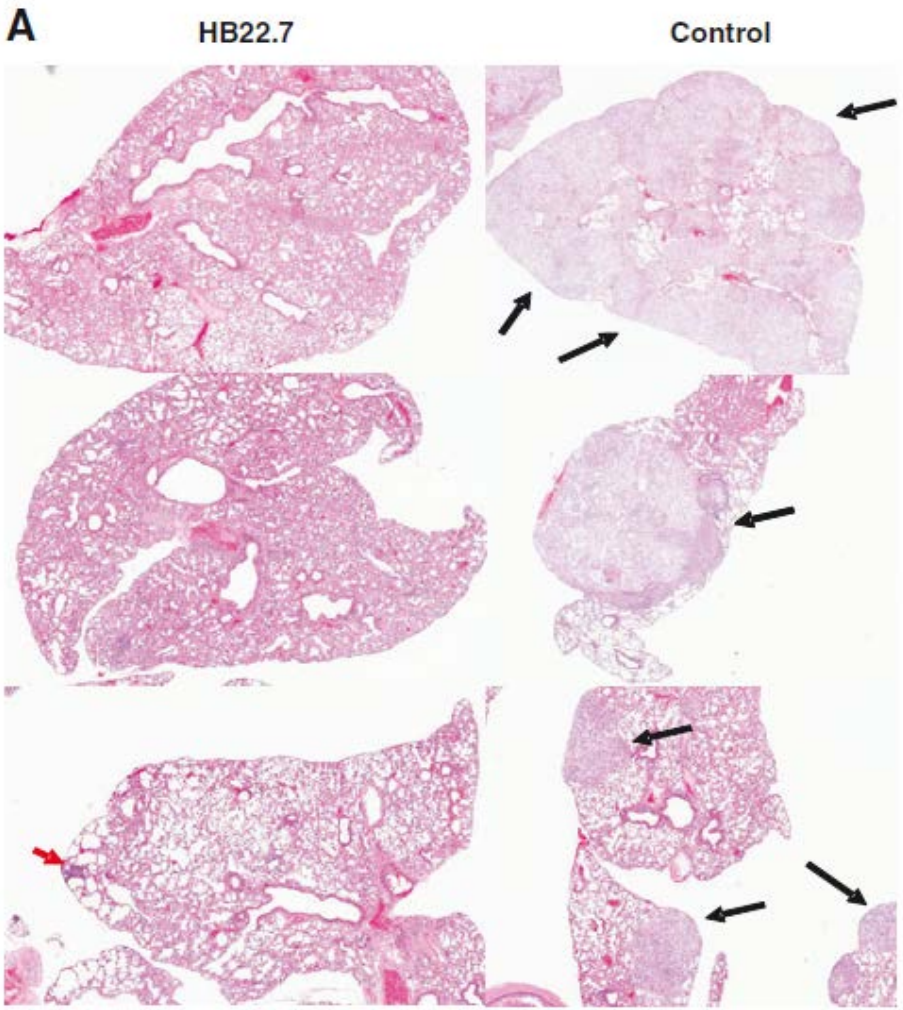
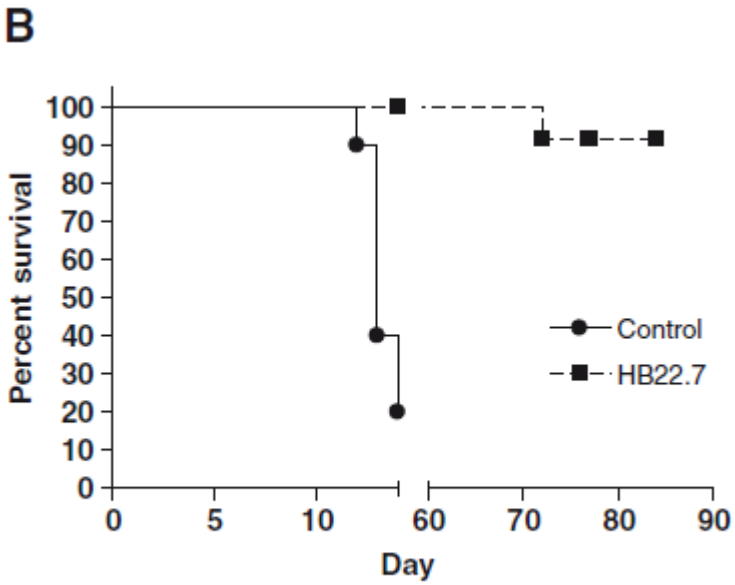
Results: : Xenograft studies – metastatic model of lung cancer





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