Immunotherapy of Breast Cancer



World Congress on Breast Cancer

Birmingham, UK

August , 2015

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Approaches to Cancer Immunotherapy

- Adoptive immunotherapy (Darcy, Beavis)
  - Augmenting the immunogenicity of patients own effector cells ex vivo (CAR T cells)
  - Passive transfer of engineered antibodies
- Active Immunotherapy (Paterson, Mason, Kieber-Emmons)
  - Augmenting the natural immunogenicity of the tumor using check-point inhibitors
  - Deliver a shared or neo tumor antigen using an immunogenic vector. “Cancer Vaccines”
- Antigen Discovery (Eichmüller, Kieber-Emmons)
  - Serves both adoptive immunotherapy (cell surface antigens)
  - And active immunotherapy for delivery as cancer vaccines or as a model for synthetic neoantigens

What is a cancer "vaccine"?

A way to harness the natural immune response to tumors by active immunization using similar strategies that have proved successful for prophylactic vaccines against infectious disease

Cancer vaccines are applied therapeutically but as we learn more about the genes that predispose individuals to specific cancers, prophylactic vaccines may also become a reality

FDA approved/accepted forms of immunotherapy for cancer

- Allogeneic effects: BMT/PBSCT and DLI
- Aldesleukin (IL-2)
- Avastin ® (Bevacizumab, anti-VEGF)
- BCG (Bacille Calmette–Guérin)
- Daclizumab® (anti-CD25 mab)
- Erbitux® (anti-EGFR)
- Herceptin® (Trastuzumab): anti-HER2/neu
- Interferon alpha (type 1 interferon)
- Ipilimumab® (anti-CTLA4)
- Keytruda® (pembrolizumab, anti-PD1)
- Mylotarg® (anti-CD33 calicheamicin immunotoxin)
- Ontak® Denileukin diftitox (anti-CD25, IL-2 receptor immunotoxin)
- Opdivo® (nivolumab, anti-PD1)
- Provenge® (pulsed DCs)
- Rituxan ® (Rituximab, anti-CD20)

HER-2/neu as a target for *Listeria*-based cancer immunotherapy.

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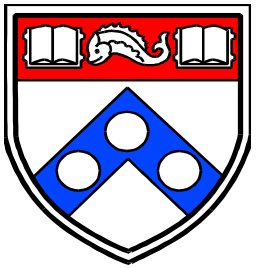
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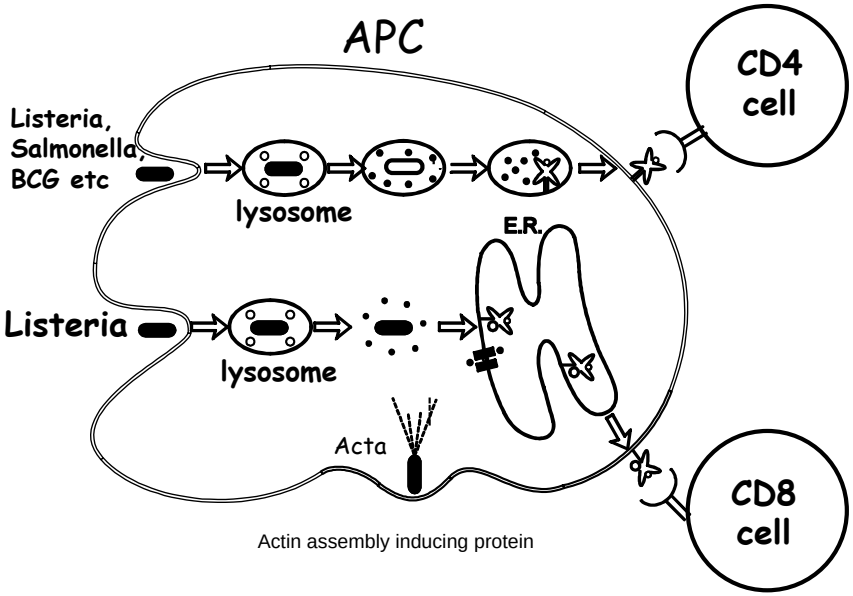


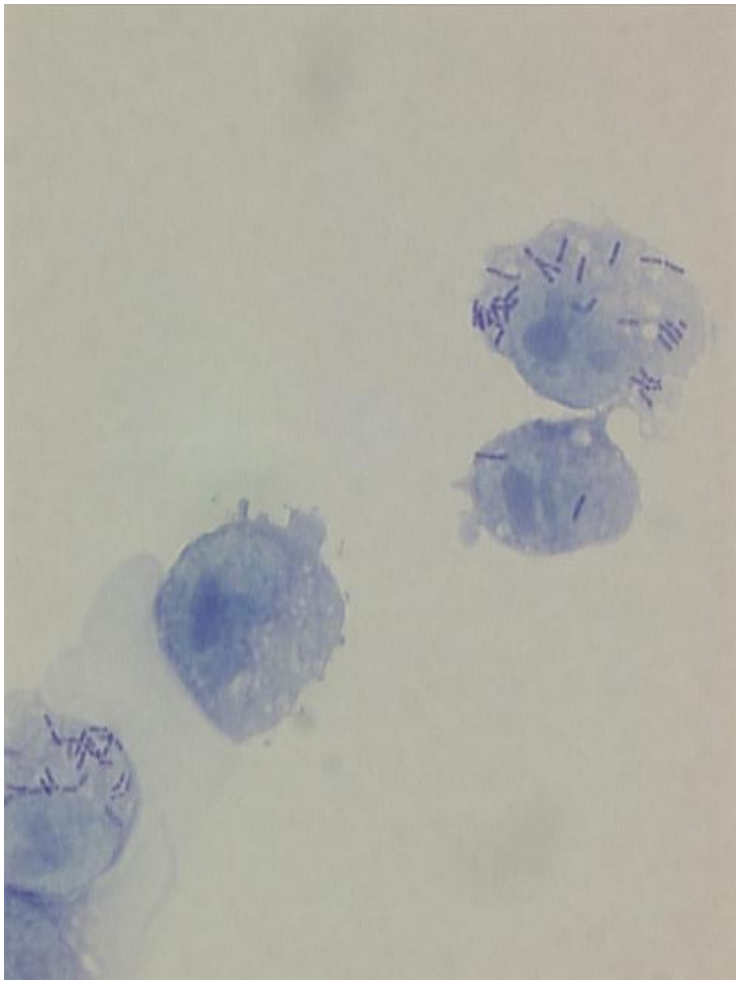
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A Unique Vaccine Vector: *Listeria monocytogenes*

- Gram positive intracellular bacteria
- Food borne pathogen that lives in the cytosol of phagocytic cells
- Unique life cycle ideal for antigen presentation
- Attenuated strains have been created that escape the vacuole



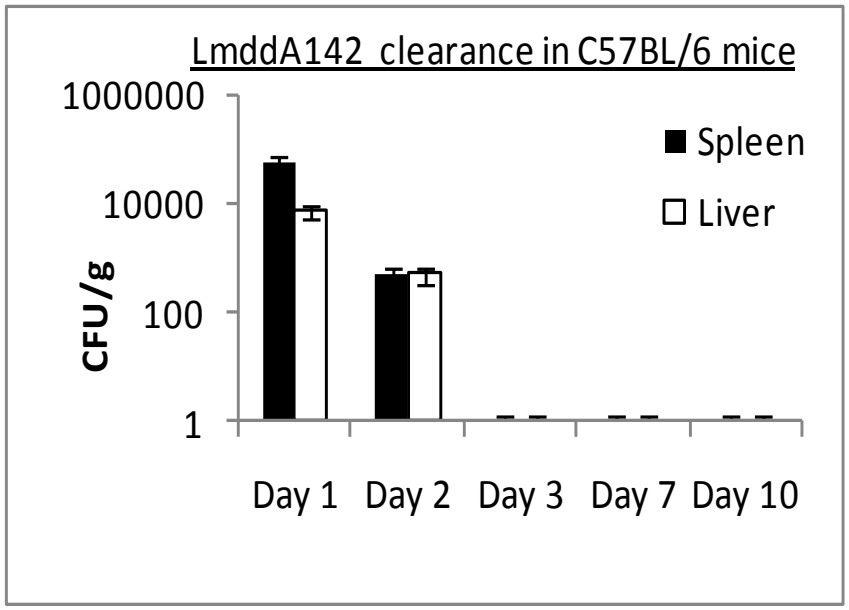


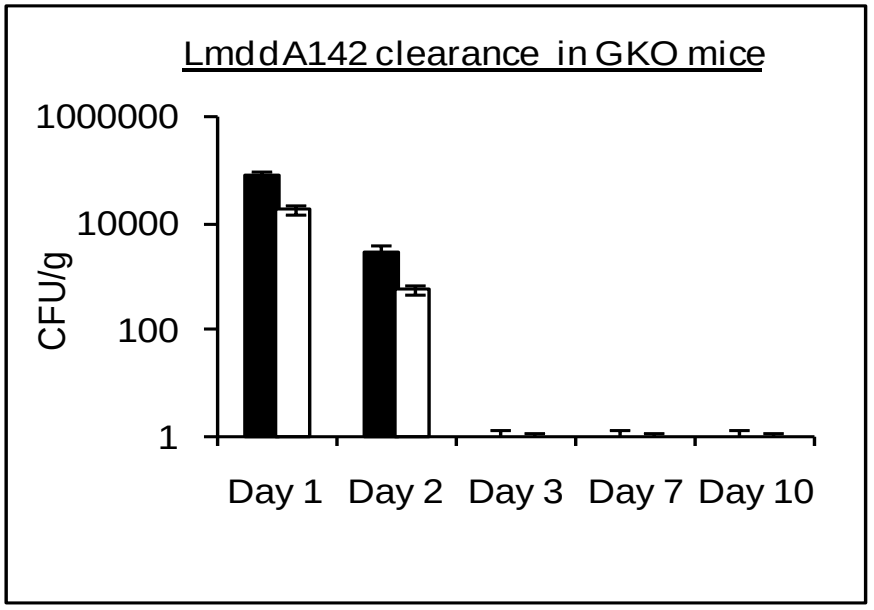
Genetic modifications of *L. monocytogenes* for use in cancer immunotherapy

Genetic modifications:

- Attenuation
  - Irreversible Act-A deletion (δAct), required for cell-to cell spread
  - Deletion of *dal* and *dat* genes required for D-alanine synthesis
- Delivery of antigenic payload
  - Plasmid based strategy
  - Antigen of interest is fused to LLO, which acts as a PAMP
  - Maintenance of plasmid through auxotrophic complementation of the *dal* gene
  - No antibiotic selection marker – safer for clinical use

In vivo clearance of attenuated *dal dat δActA* 142 (LmddA142) strain





$1x10^8$ CFU administered i.p. in C57BL/6 and IFN-γ knock out (GKO) mice. (Limit of detection is 100 CFU)

Wallecha, A., et al. 2009. Clinical & Vaccine Immunol. 16(1):96-103

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Lm-LLO Vaccines: effects on innate immunity

- Infect APC & other myeloid cells to generate a very strong innate immune response
  - SCID mice clear attenuated Listeria strains via innate immunity
  - Th-1 pattern of cytokine & chemokine release
  - Up regulation of co-stimulatory molecules including: CD 25, CD40, CD 80 (B7.1), CD 83, CD 86 (B7.2), B7-H1 (PD-L1), B7-DC (PD-L2)
  - Cytokines(IL-6, IL-12, IL-18,TNF-alpha, GM-CSF) released from infected myeloid cells early in infection stimulate NK cells to release IFN-γ and promotes the maturation of DCs

Lm-LLO Vaccines: effects on adaptive immunity

- Activates strong CTL cell response even in the face of tolerance
- Induces Th1 cells that secrete IFN-γ
- IFN-γ stimulates tumor cells to produce CXCL-9 and -10 that recruit CXCR3 expressing CTL.
- Induces $T_{\gamma\delta}$ associated with IL-17 secretion
- Minimizes inhibitory T cells (Tregs) and inhibitory cytokines, TGF-beta and IL-10
- Appears to reduce the number of MDSC within tumors but not in the periphery

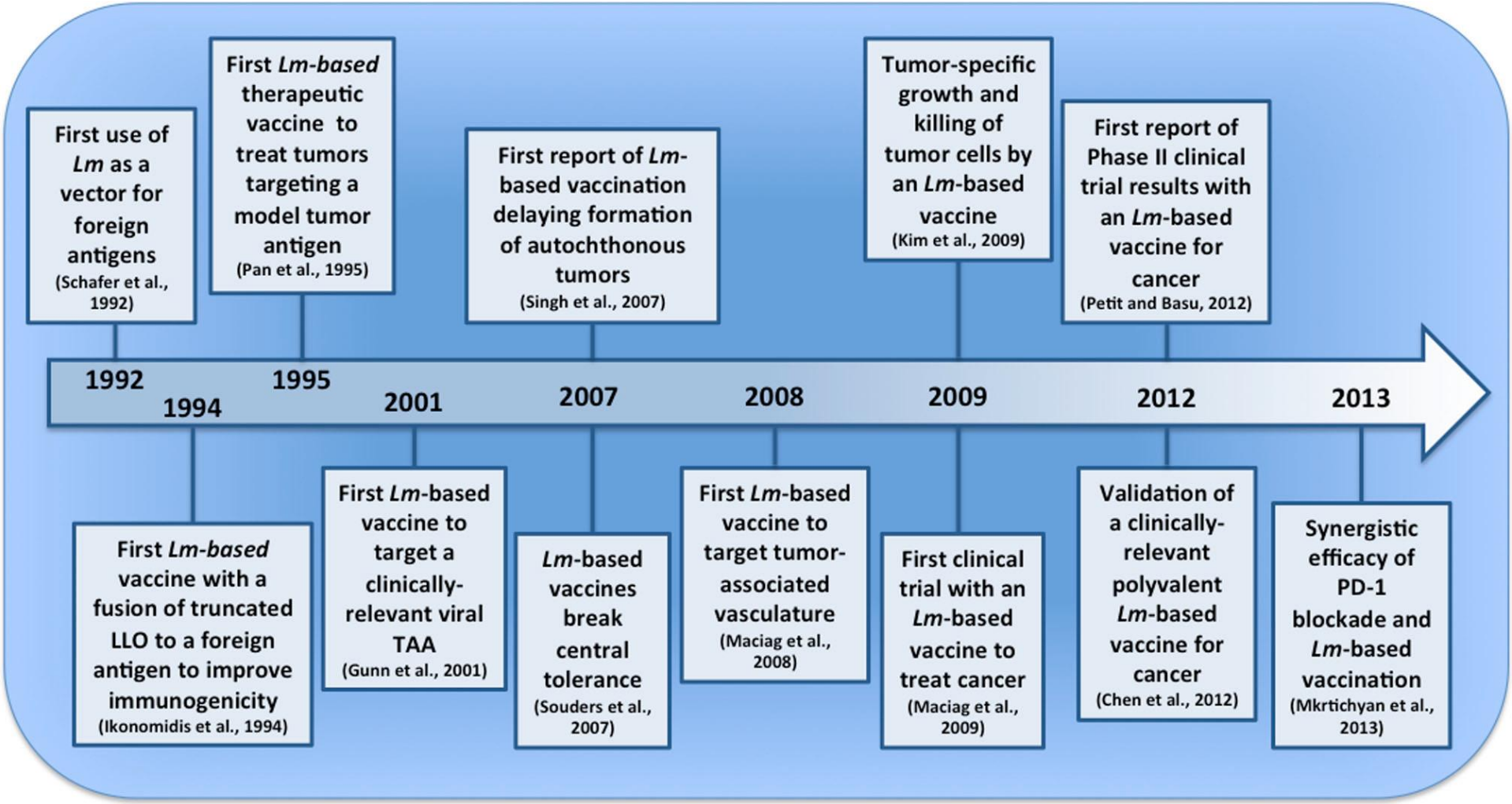
Live *Listeria* Vaccines: tapping into evolution

- By redirecting listerial immunity against cancer we are harnessing mechanisms that have evolved over millennia and are infinitely more complex than anything we know how to build.

- Unlike many other therapies that focus on one or two immune mechanisms, live *Listeria* vaccines have many independent mechanisms of action that are coordinated, simultaneous and integrated.

- In addition, *Listeria* invades tumors either directly or by infecting tumor infiltrating cells, which creates a local tumor micro-environment that supports anti-tumor efficacy and direct killing.

Pivotal, published pre-clinical events in the development of Lm-based vectors for tumor immunotherapy



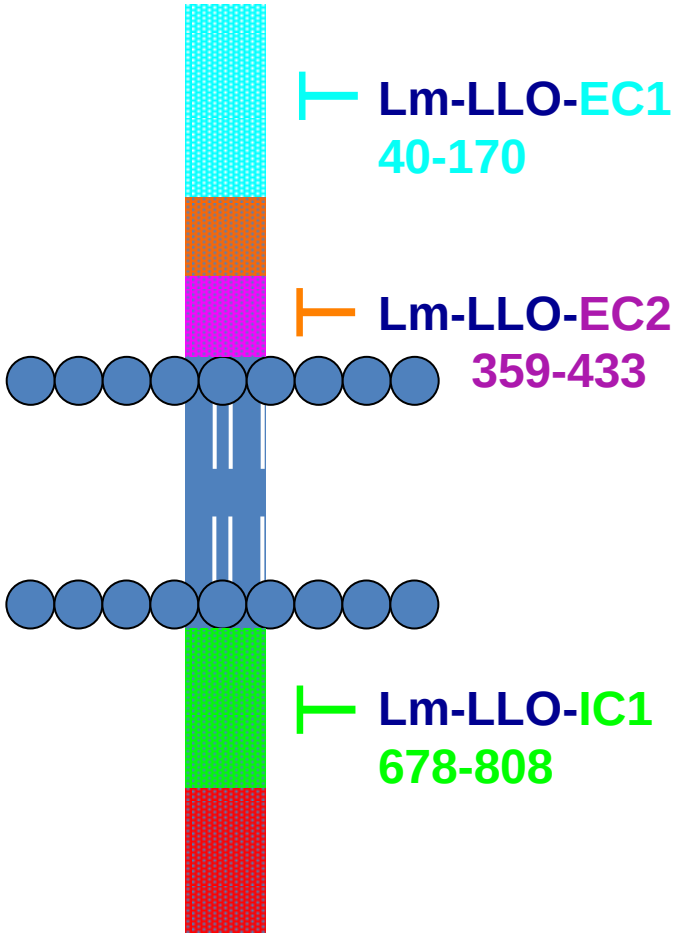
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Targeting the tumor antigen HER-2/neu using *Listeria monocytogenes* for the immunotherapy of breast cancer and osteosarcoma

Her2/neu (erbB2)

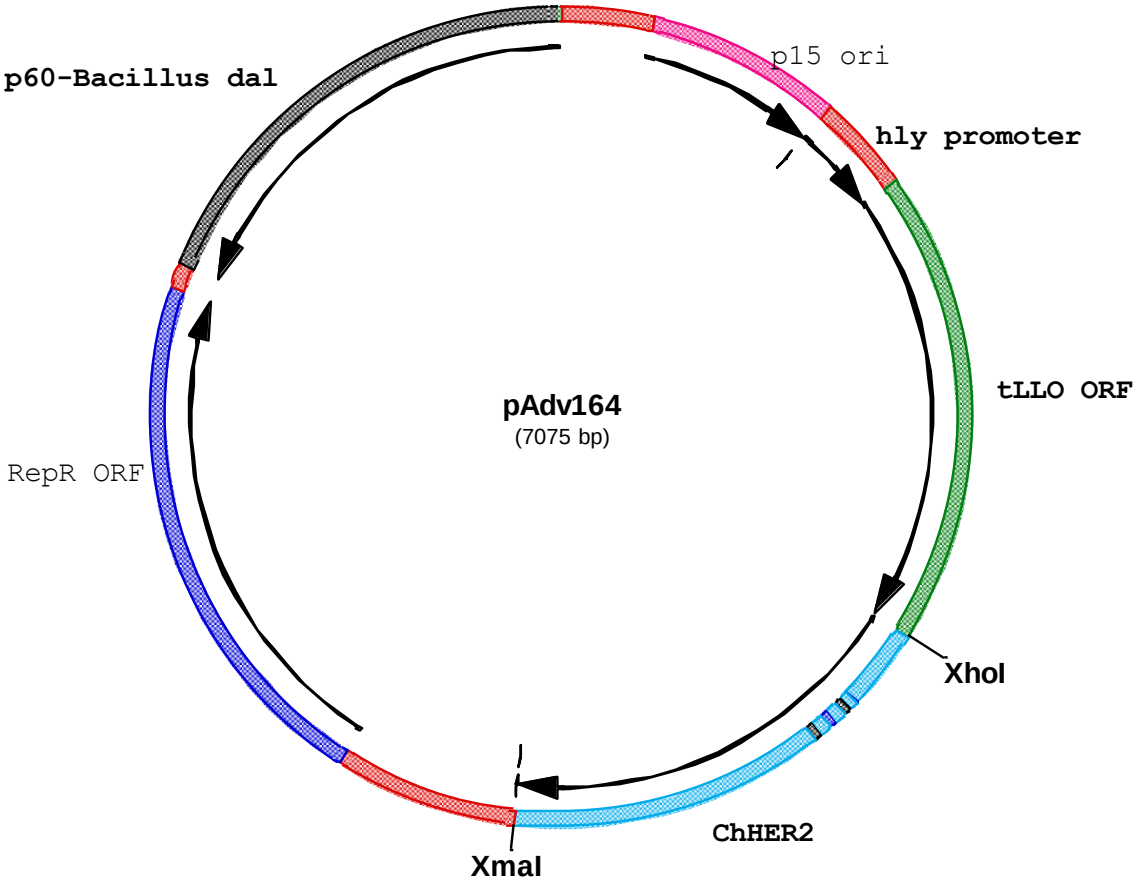
- Large transmembrane protein (1260 residues)
- EGFR family
- Expressed during development and differentiation
  - Skin, mammary glands, cardiac tissue, neuronal tissue
- When mutated it forms heterodimers with erbB1, erbB3 and erbB4 – stabilizes dimers, reduces ligand dissociation, promoting strong and prolonged downstream signaling
- Activates multiple intracellular signaling pathways – PI3K and MAPK
- Promotes cellular proliferation and survival, angiogenesis
- Amplification and over-expression of Her2/neu:
  - Carcinomas: breast, ovarian, pancreatic, colorectal, stomach, prostate, HNSCC, and OSA
  - Correlates with aggressive phenotype, increased metastatic risk, and poor prognosis

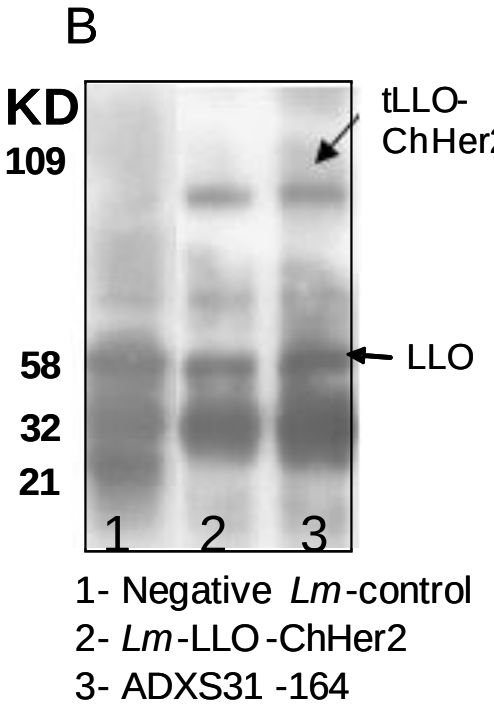
Anti-human-HER-2/neu Lm-based vaccine design



The vaccine expresses a chimeric molecule, of 458 residues, that spanned two regions in the extracellular (EC1 and EC2) and the tyrosine kinase region of the intracellular (IC1) domain, fused to LLO. These regions include all known human epitopes.

Vector design ADXS-31-164 (Lm-LLO-ChHer2).Targets multiple regions of HER-2/neu

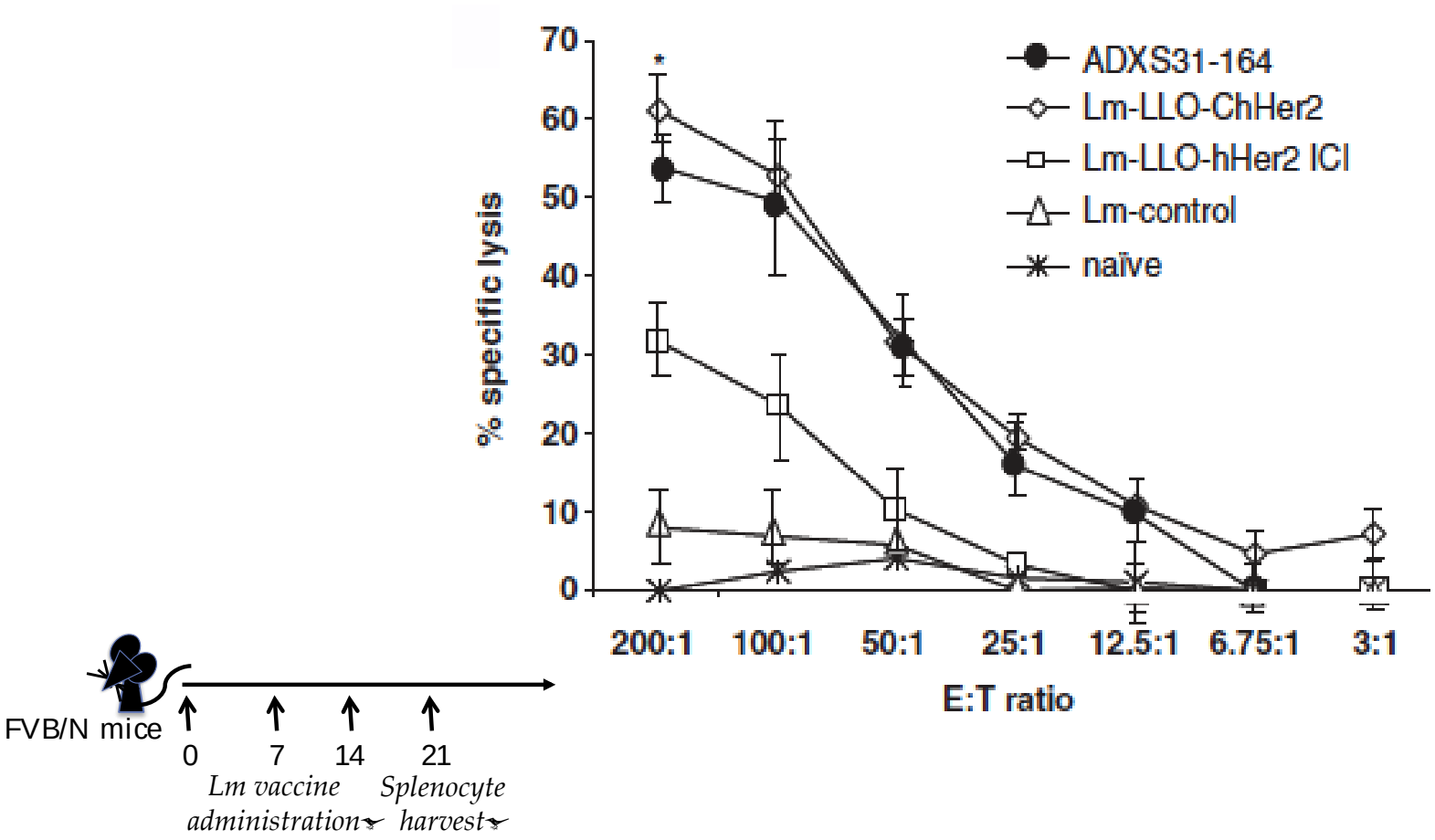




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Shahabi V., Seavey MM., et al. 2011. Cancer Gene Therapy. 18:53.

ADXS31-164 induces CTL responses in Her2+ tumors in mice



Shahabi et al. Cancer Gene Therapy (2011)

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FVB/N HER-2/neu Transgenic Mouse Model

- Rat HER-2/*neu* gene under the control of the mouse mammary tumor virus (MMTV) promoter.
  - Muller, WJ. 1991. *J Immunol 170:4273.*
  - Guy, CT *et al.* 1992. *PNAS 89:10578.*
- Express the proto-oncogene in breast tissue and hematopoietic cells.
- Slow progression of disease with metastasis to lungs and liver.
- 100% of females develop tumors between 4-6 months of life.
- Show evidence of T cell tolerance to HER-2/*neu.*

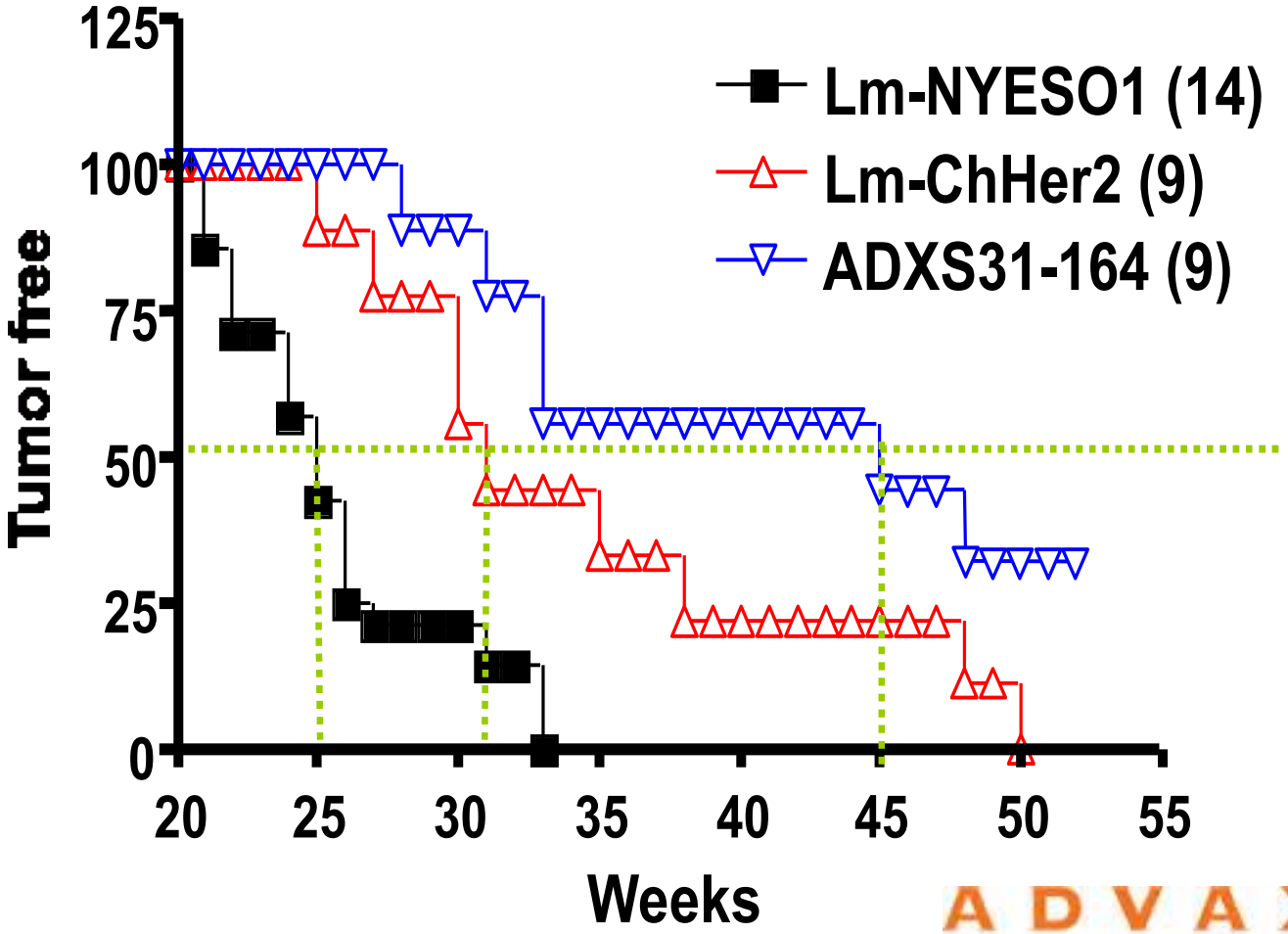
Autochthonous tumor protection

- Can we prevent or delay the onset of spontaneous tumor growth in the transgenic mice?

- Vaccinate the mice every 3 weeks for a total of 5 vaccinations starting at 6 weeks of age.
  - 6, 9, 12, 15, 18 and 21 weeks of age

- Observe appearance of tumors over the space of a year.

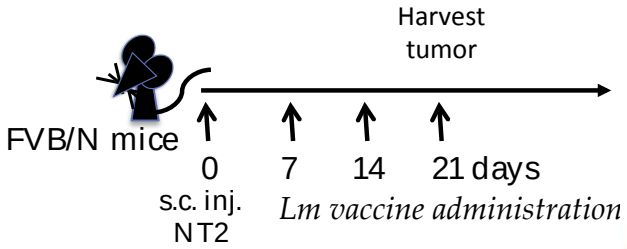
ADXS-31-164 (Lm-LLO-ChHer2). Breaks tolerance in HER-2/neu transgenic mice and delays the onset of autochthonous tumors



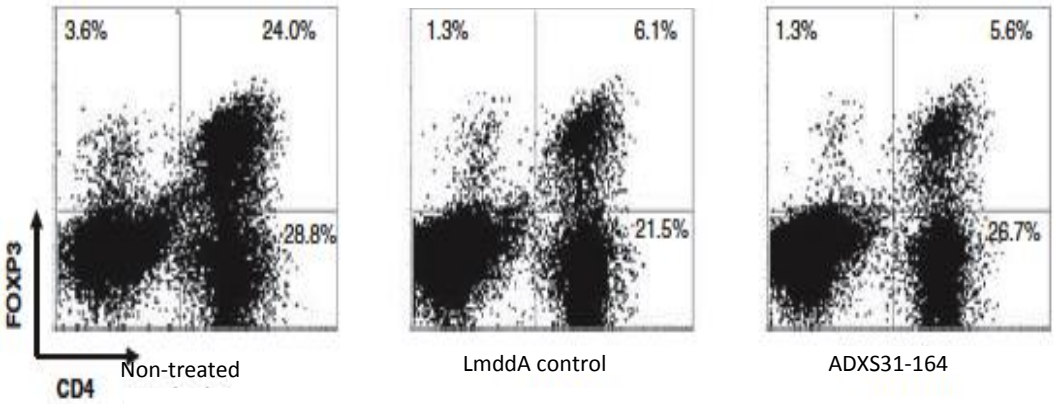
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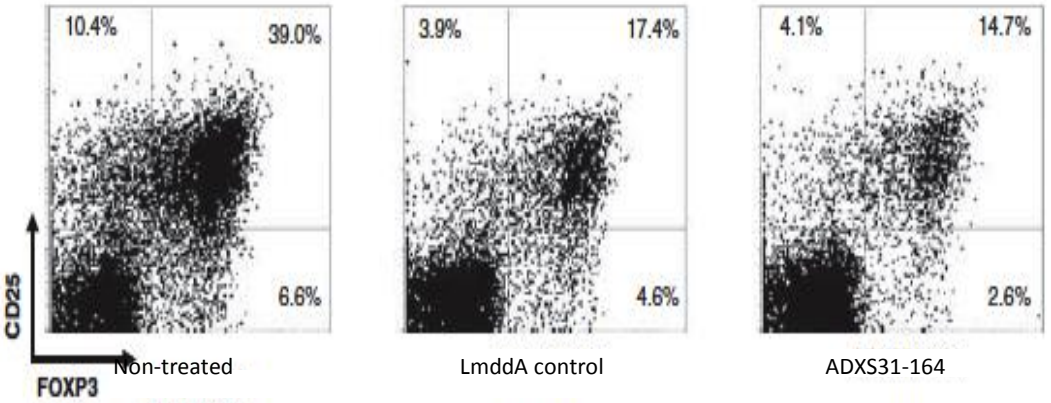
ADXS31-164 reduces Tregs in Her2+ tumors in mice



Gated on CD3+ T cells



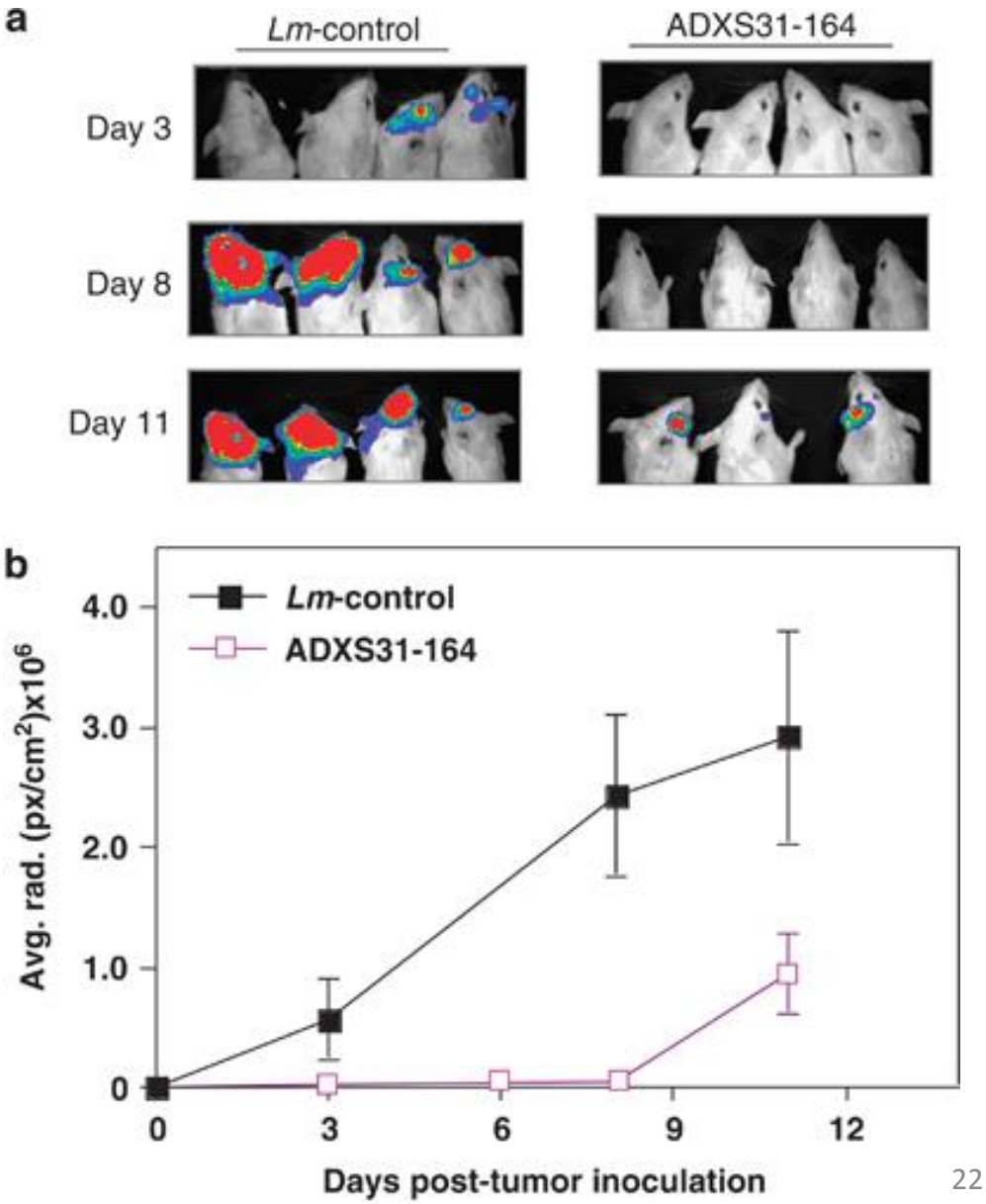
Gated on CD3+CD4+ T cells



Shahabi et al. Cancer Gene Therapy (2011)

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Vaccination with ADXS31-164 can delay the growth of a breast cancer cell line in the brain.



Targeting HER-2/neu for cancer in humans

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- HER2 is expressed in a percentage of solid tumors such as breast (25 - 40 %), gastric, bladder, brain, pancreatic, ovarian and pediatric bone cancer (osteosarcoma).
- ACS estimates that in 2015 in the United States alone there will be 231,840 new cases of invasive breast cancer; 24,590 new cases of gastric cancer; 74,000 new cases of bladder cancer; 22,850 new cases of brain/spinal cancer; 48,960 new cases of pancreatic cancer; 21,290 new cases of ovarian cancer; and 207 new cases of pediatric osteosarcoma.
- HER2 expression is associated with more aggressive disease, increased risk of relapse and decreased overall survival.
- ADXS-HER2 received orphan drug designation by the U.S. Food and Drug Administration (FDA) for osteosarcoma in May 2014.
- IND for Lm-LLO-cHER-2/neu (ADXS31-164) for multiple HER-2/neu overexpressing tumors including breast cancer approved in January 2015
- Phase 1 clinical trial about to begin recruiting patients with HER-2 expressing solid tumors.

Clinical Development: ADXS-PSA and ADXS-HER2

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Product		Indication	Phase 1	Phase 2	Phase 3
ADXS-PSA		**Prostate Cancer**			
	M C	Metastatic – Combo with KEYTRUDA® [1](pembrolizumab)	Phase 1/2		
ADXS-HER2		**HER2-positive Solid Tumors (including Osteosarcoma*)**			
	M	Metastatic – Single Arm	Phase 1		
	M	Pediatric Osteosarcoma (Planned with COG)		Phase 2	

M Monotherapy
C Combination

Completed
In Process
Planned 2015
In Process = FDA accepted IND and/or ongoing trial

1 Partnership with Merck
* Orphan Drug Designation

Clinical Development: ADXS-HPV

ADVAXIS IMMUNOTHERAPIES™

Product		Indication	Phase 1	Phase 2	Phase 3
ADXS-HPV		**Cervical Cancer***			
	M	AIM2CERV – Adjuvant Randomized vs Placebo			Phase 3
	M	Metastatic – Randomized vs Cisplatin/ADXS-HPV	Phase 1	Phase 2	
	M	Metastatic – GOG		Phase 2	
	M	Metastatic – Single Arm High Dose	Phase 1/2		
	C	Metastatic – Combo with MEDI 4736[1]	Phase 1/2		
	C	Stage I-IIa – Combo with epacadostat (INCB24360) [2]		Phase 2	
		Head and Neck Cancer*			
	M	Neoadjuvant – Window of Opportunity - Mount Sinai		Phase 2	
	C	Metastatic – Combo with MEDI 4736[1]	Phase 1/2		
		Anal Cancer*			
	M	RTOG – Adjuvant Randomized vs Control		Phase 2/3	
	M	Adjuvant – Single Arm High Risk – Brown University (BrUOG)	Phase 1/2		
	M	Metastatic – Single Arm		Phase 2	

M Monotherapy
C Combination

Completed | In Process | Planned 2015

In Process = FDA accepted IND and/or ongoing trial

1 Partnership with MedImmune (AZ)
2 Partnership with Incyte
* Orphan Drug Designation

Targeting human HER-2/neu for cancer in dogs – why dogs?

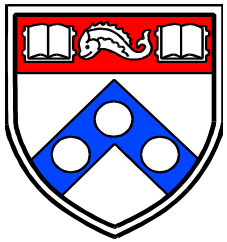
- They are not mice! They are large, outbred omnivores – just like humans. They also have a similar digestive system and human-like E-cadherin.

- Human HER-2/neu is 92% homologous to canine HER-2/neu but only 87% homologous to rodent HER-2/neu but human HER-2/neu is effective against Her-2/neu expressing tumors in mice.

- 40-60% of canine and human osteosarcoma express HER-2/neu

- People love their dogs!

Acknowledgements



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