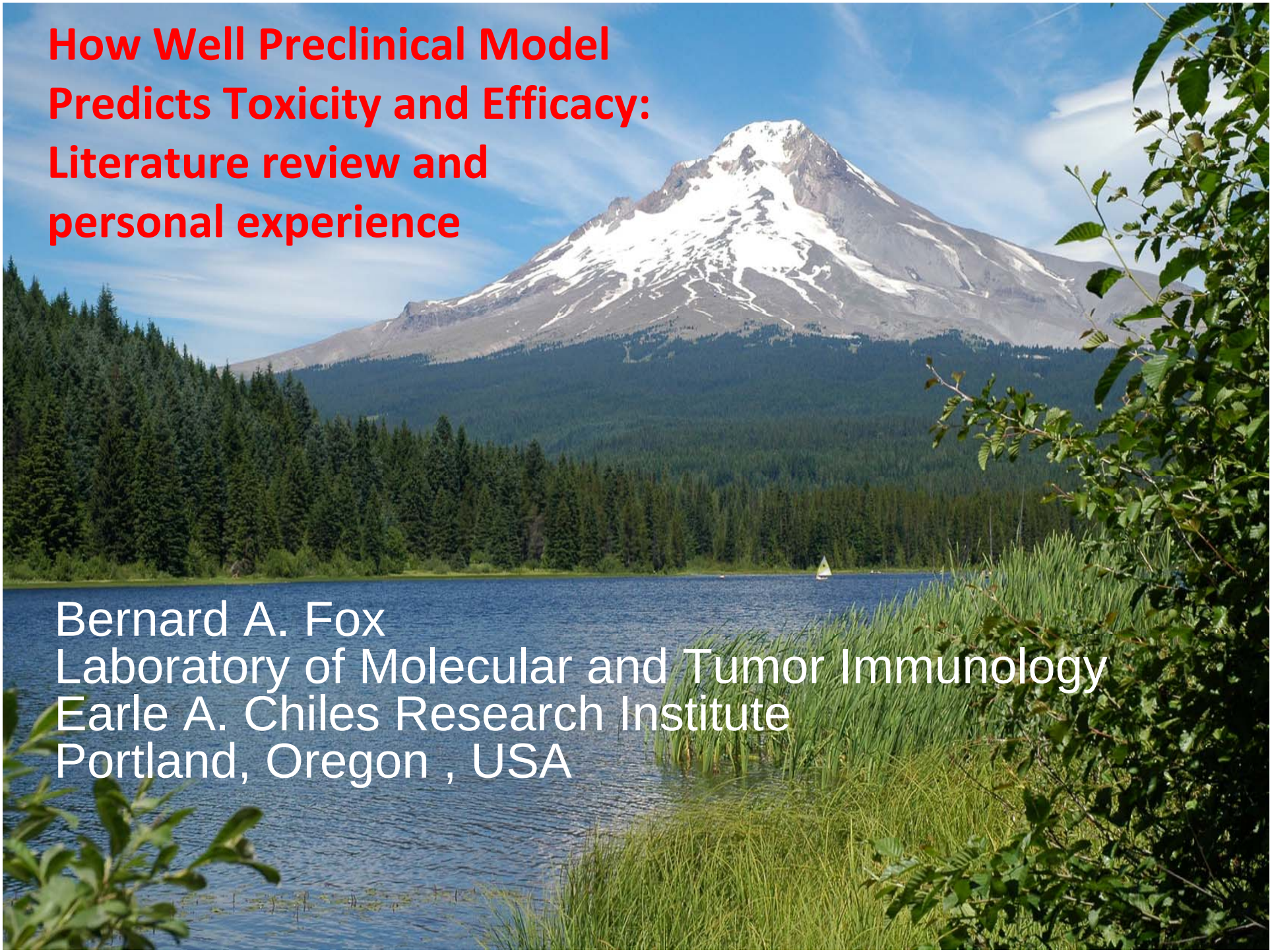
How Well Preclinical Model Predicts Toxicity and Efficacy: Literature review and personal experience
Bernard A. Fox
Laboratory of Molecular and Tumor Immunology
Earle A. Chiles Research Institute
Portland, Oregon , USA

I have Consultant/Advisory Roles or Research support/Grant to disclose.

BMS, MannKind, BioSante (Cell Genesys),

Dendreon, Immunophotonics, MannKind, MicroMet (Amgen), Ventana/Roche,

Yes, I have a Leadership Position and Stock Ownership to disclose.

UBIVAC

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Guidance for Industry

Clinical Considerations for Therapeutic Cancer Vaccines

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
October 2011

Safety and Activity of Multi-antigen vaccines

- Cancer vaccine formulations may contain multiple tumor-associated antigens in order to generate multiple tumor-specific immunologic responses and potentially hinder potential tumor escape mechanisms.
- In general, each component of a multi-antigen vaccine may not need to be individually evaluated for safety and activity, this will be considered on a case-by-case basis.

FDA 2011

Presence of the target antigen in normal tissues

- Since potential vaccine-related toxicities may be related to the presence of:
- Target antigen in normal tissues, or
- Unrelated protein in normal tissues that may contain a peptide sequence similar to a peptide in the vaccine, the presence of target antigen in normal human tissues should be determined.

- For peptide vaccines, sequence homology searches should be conducted to assist in prediction of potential vaccine-related toxicities.

FDA 2011

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Why do preclinical models?

- Are their data to suggest that preclinical models have potential to inform about possible toxicity?

Why do preclinical models?

- Are the data to suggest that preclinical models have potential to inform about possible toxicity?

Cancer Vaccines:

Similar to human data (Khleif and colleagues), cancer vaccines (preclinical) have provided little evidence of toxicity (with exception of vitiligo).

Review article

Autoimmunity associated with immunotherapy of cancer

*Sally M. Amos,[1] *Connie P. M. Duong,[1] Jennifer A. Westwood,[1] David S. Ritchie,[2] Richard P. Junghans,[3] †Phillip K. Darcy,[1,4,5] and †Michael H. Kershaw[1,4,5]

BLOOD, 21 JULY 2011 • VOLUME 118, NUMBER 3

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Autoimmunity associated with immunotherapy of cancer

Table 1. Immune-mediated toxicities associated with adoptive immunotherapy of cancer

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CAR-modified T cells		VEGF-R2	Various	Various organs, probably the result of cytokine-induced hypotension
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Adoptive immunotherapy with high avidtity/affinity TCR/CARs may have significant therapeutic efficacy.

But they may also have substantial toxicity

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Cancer Vaccines

- Historically - lack of efficacy of cancer vaccines **against established tumors**.
- Limited toxicity (vitiligo).
- No therapeutic activity.

Cancer Vaccines / Combination Immunotherapy

- Therapeutically effective..
- But will they now also generate toxicity?

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CEA-Tg mice

- These mice contain the human CEA transgene under the control of the endogenous human CEA promoter
- Express CEA in normal gastrointestinal tissue and in fetal tissue in a manner similar to that expressed in humans .
- mice are tolerant to CEA by their inability to mount either CEA-specific T-cell responses or CEA-specific antibody responses after multiple vaccinations with CEA protein in adjuvant

Clin Cancer Res 2003;9:1837-1849.

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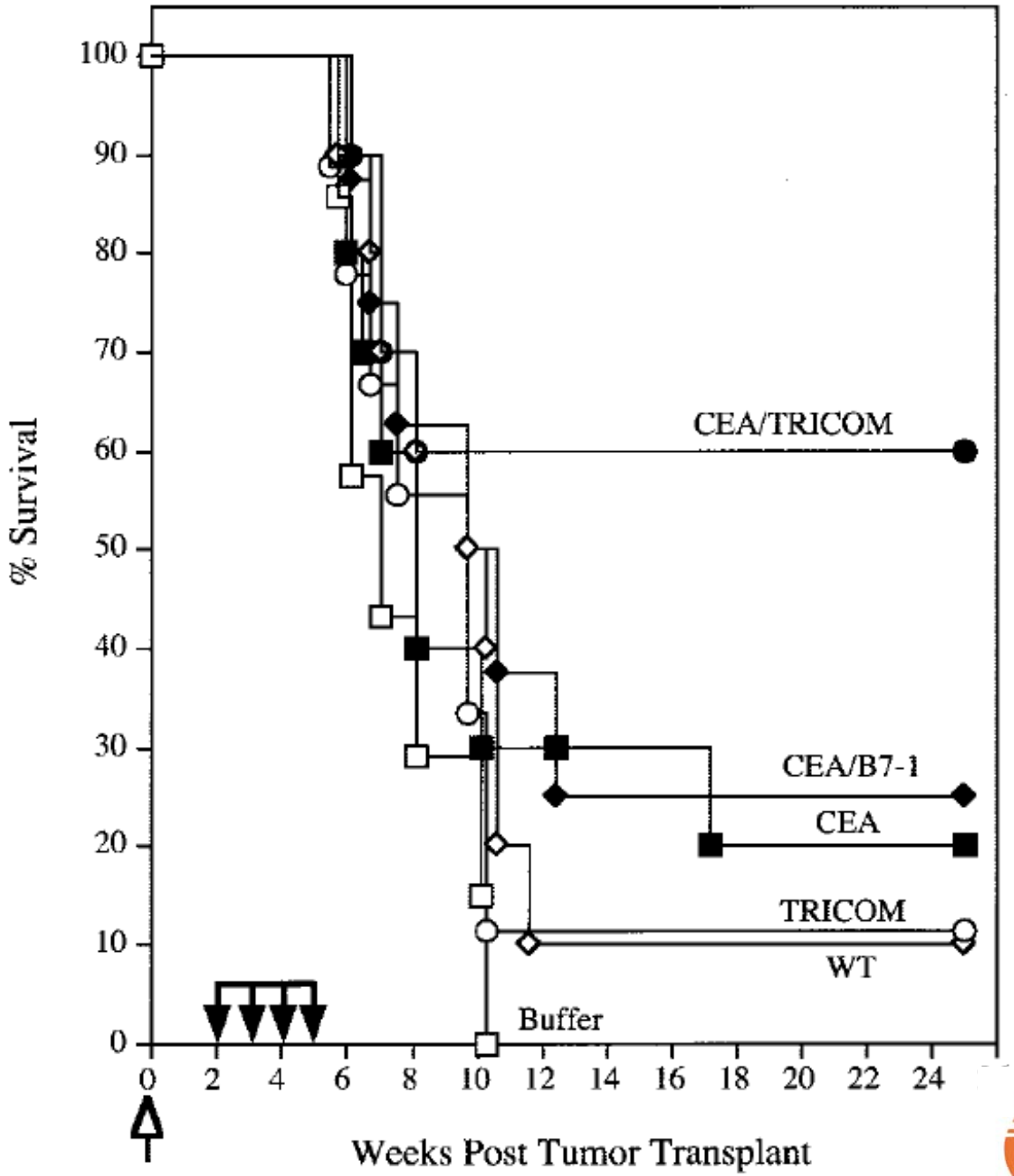
Vaccine Therapy of Established Tumors in the Absence of Autoimmunity

James W. Hodge, Douglas W. Grosenbach, Wilhelmina M. Aarts, Diane J. Poole, and Jeffrey Schlom[1]

Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland 20892-1750

Clin Cancer Res 2003;9:1837-1849.



Tricom vaccine

Two week Established tumor

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Table 2 Serum immunological parameters of CEA-Tg mice that were cured of tumor with CEA/TRICOM vectors

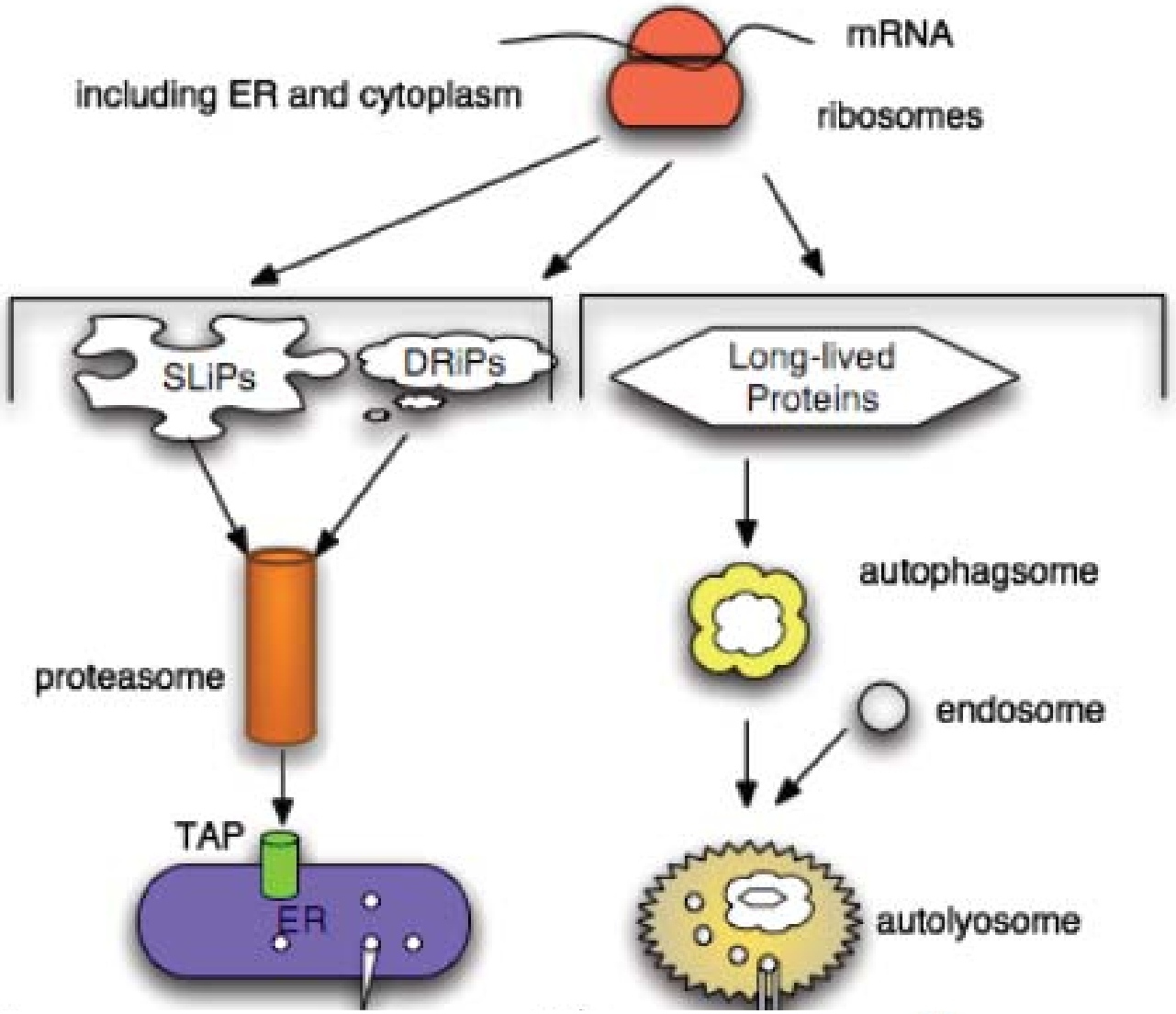
Test	Normal	Treatment group[a] ($n = 5$)	Age-matched control group[b] ($n = 5$)
CEA Protein	13–100 ng/ml	37.1 ± 12.1	24.3 ± 11.2
CEA Ab[c]	<50	1640 ± 250	<50
Vaccinia Ab[c]	Negative	200	<50
Fowlpox Ab[c]	Negative	150	<50
B7-1 Ab[d]	Negative	<100	<100
ICAM-1 Ab[d]	Negative	<100	<100
LFA-3 Ab[d]	Negative	<100	<100
GM-CSF Ab[e]	Negative	Negative	Negative
nRNP Ab[e]	Negative	Negative	Negative
Histone Ab[f]	Negative	Negative	Negative
Scl-70 Ab[f]	Negative	Negative	Negative
dsDNA Ab[f]	Negative	Negative	Negative
ssDNA Ab[f]	Positive[g]	Positive (2+)	Positive (1+)
CIC[f]	Negative	Negative	Negative

Cancer Vaccines / Combination Immunotherapy

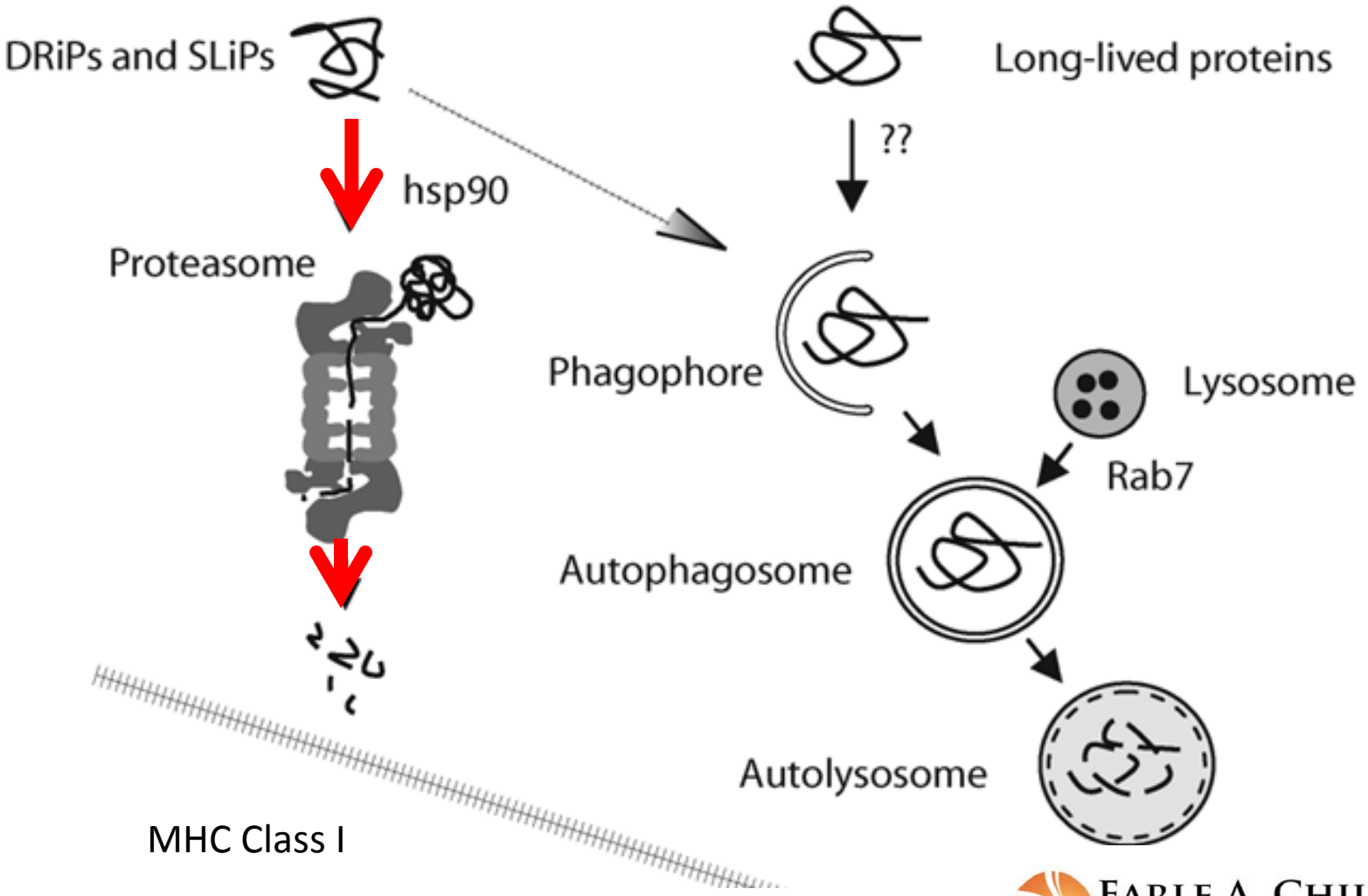
- New class of vaccines - Autophagosomes / DRibbles
  - Contain SLiPs and DRiPs
  - DAMPS, HSP, multiple TLR agonists

- Therapeutic?
- Toxicity?

Two Protein Degradation Pathways



DRiPS and SLiPS Rapidly Degraded by Proteosome and Bound by MHC Class I



Li, Y., et al., Autophagy 5:4, 576-577 2009

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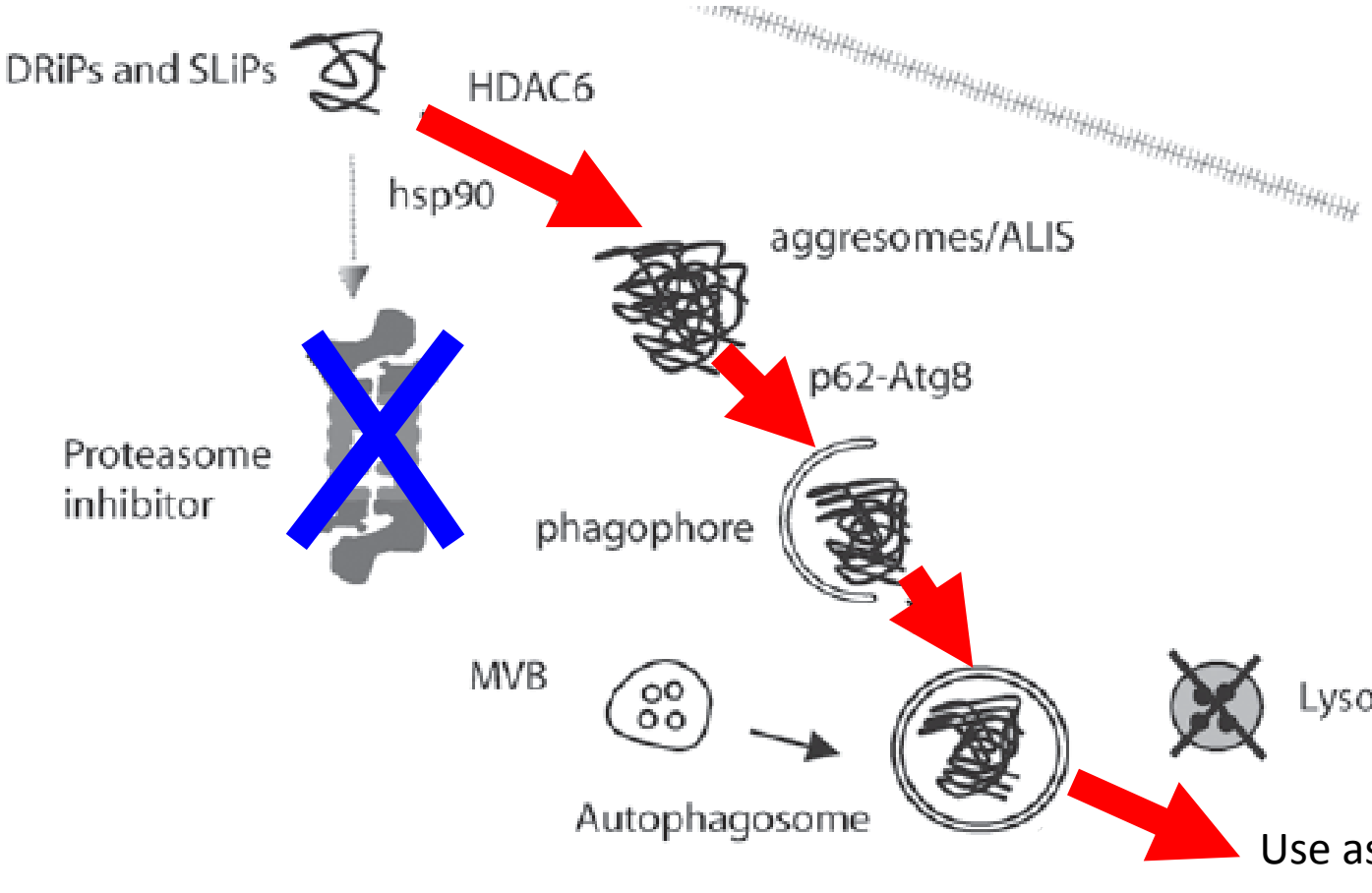
Proteosome Blockade shunts DRiPS and SLiPS to Autophagy Pathway



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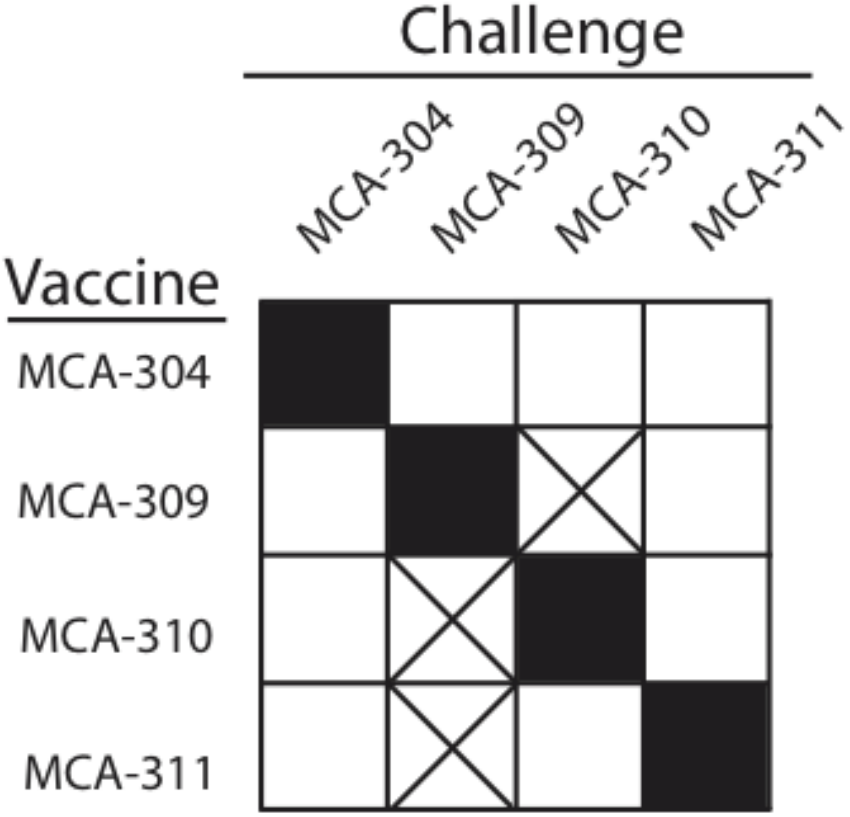
Whole tumor cell vaccines fail to protect against independently-derived syngeneic sarcomas

Whole Cell Vaccine



Twitty C., et al., Clin Can Res., 17:6467, 2011

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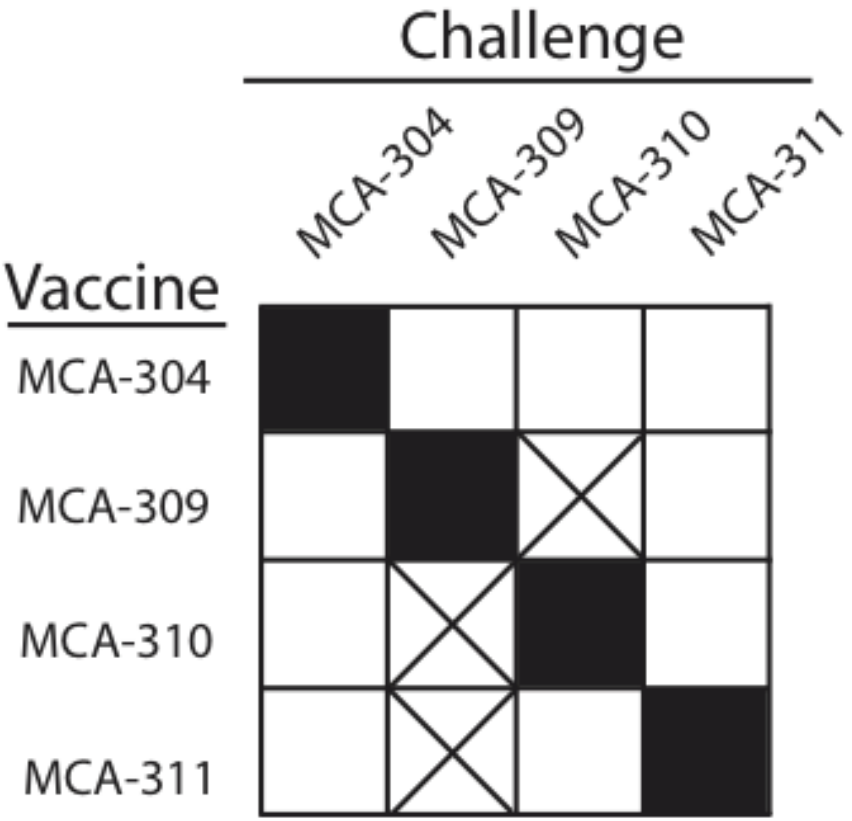
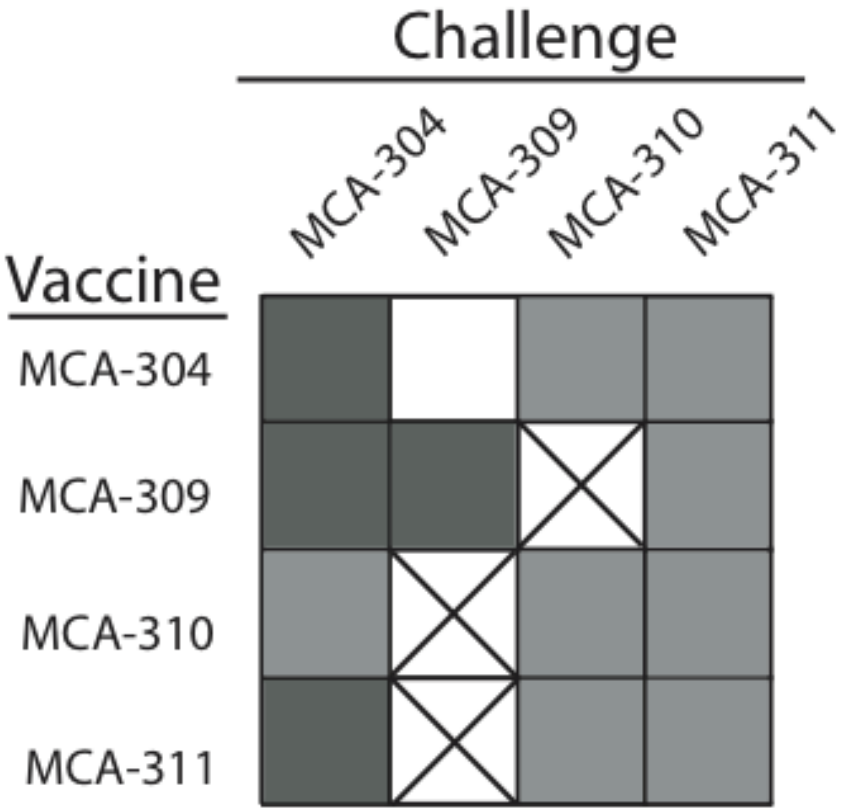
Autophagosomes from one unique sarcoma can prime an immune responses against other independently-derived syngeneic sarcomas

Whole Cell Vaccine



Autophagosome Vaccine



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Cancer Therapy: Preclinical

Clinical Cancer Research

Tumor-Derived Autophagosome Vaccine: Induction of Cross-protective Immune Responses against Short-Lived Proteins through a p62-Dependent Mechanism

Chris Twitty[1,2], Shawn M. Jensen[1], Hong-Ming Hu[1,3], and Bernard A. Fox[1,2]

Cancer Therapy: Preclinical

Clinical Cancer Research

Tumor-Derived Autophagosome Vaccine: Mechanism of Crosspresentation and Therapeutic Efficacy

Yuhuan Li[1], Li-Xin Wang[1,6,7], Puiyi Pang[1], Zhihua Cui[1], Sandra Aung[5], Daniel Haley[3], Bernard A. Fox[2,4], Walter J. Urba[4], and Hong-Ming Hu[1,4,6]

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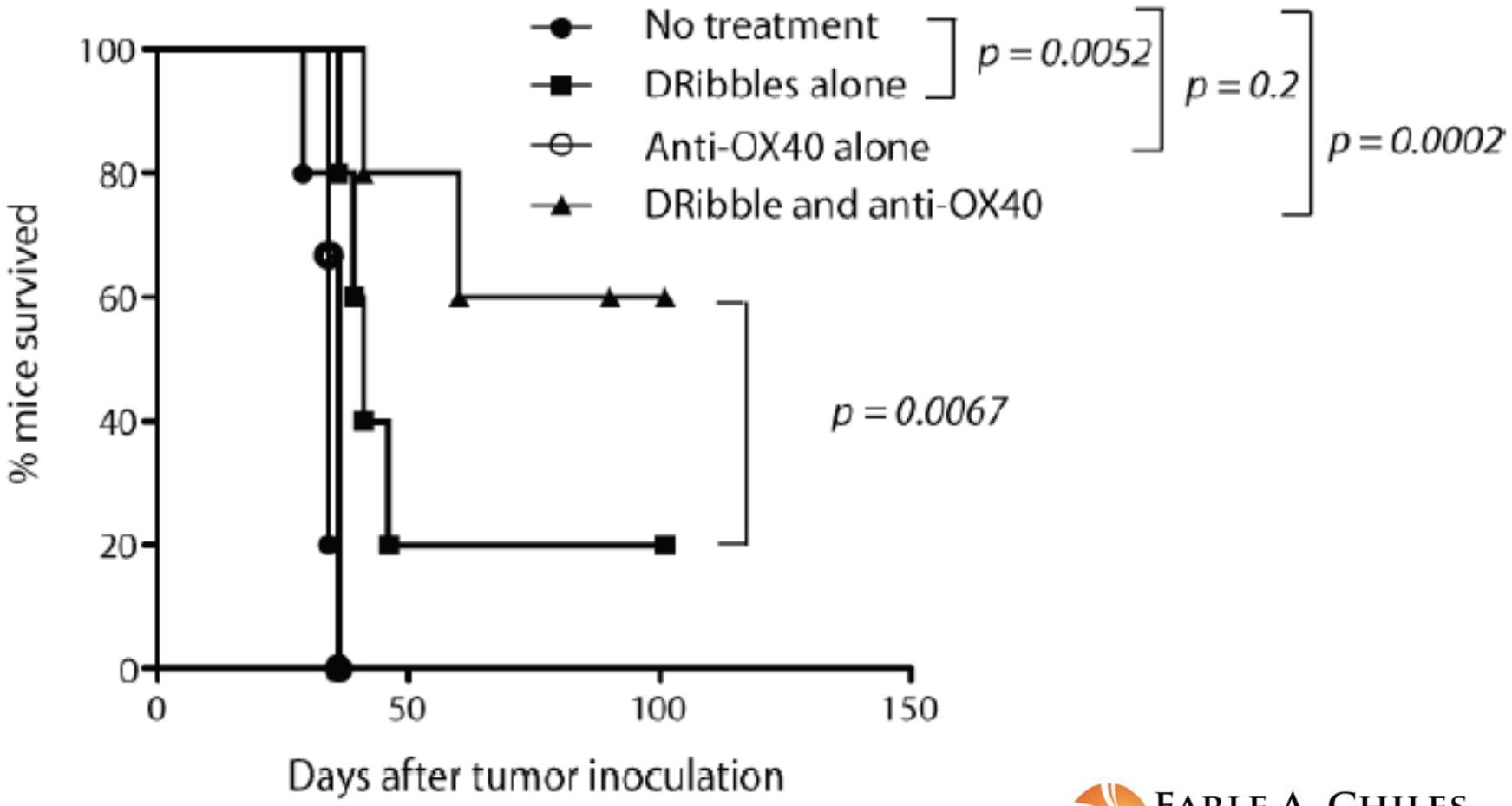
13 day therapeutic intranodal DRibble vaccine



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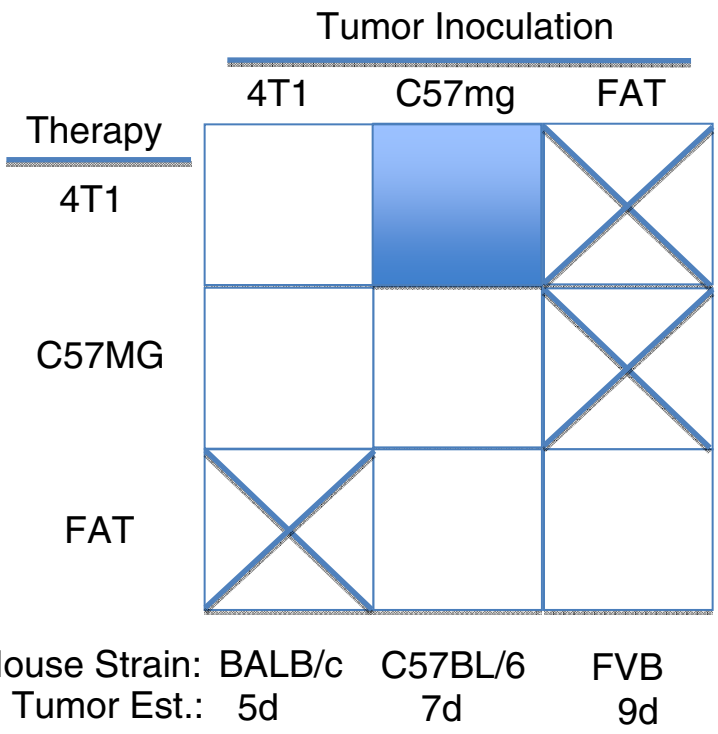
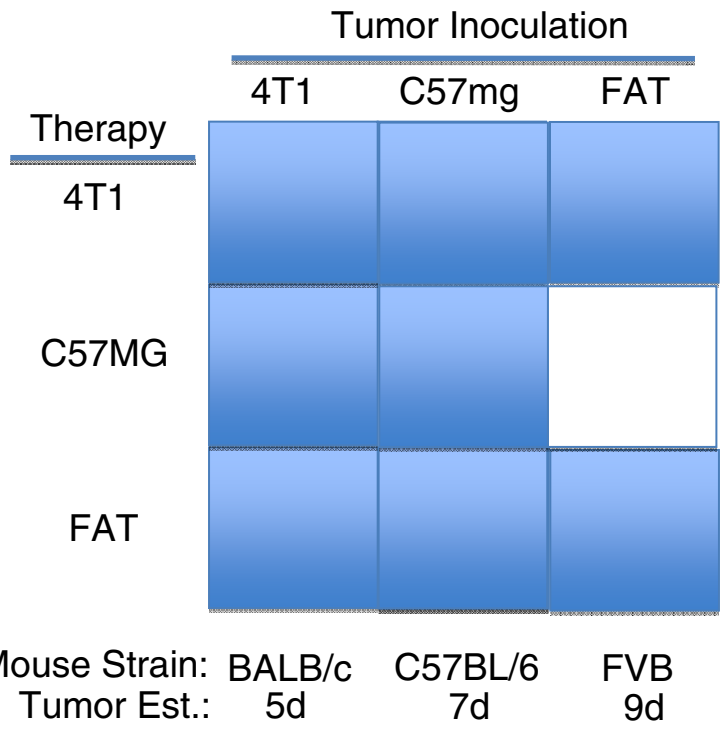
Therapeutic immunity in three allogeneic tumor models using DRibbles but not whole cell vaccines

Whole Cell Vaccine



DRibble Vaccine



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DRibble vaccine provides therapeutic efficacy without autoimmunity

- Summary of 51 therapy and 16 protection experiments:
  - 226 mice were long-term survivors
  - DRibble vaccine from one of 11 different tumors
  - 176 received an intranodal DRibble vaccine
  - 56 received DRibbles pulsed onto DC (protection experiments).

- Sarcomas (n=4), mammary cancer (n=4), melanoma (n=1), renal (n=1) or prostate cancer (n=1).

- We have observed therapeutic or protective effects with each vaccine, but **have not observed clinical signs of toxicity**

Cancer Vaccines / Combination Immunotherapy

- Can have therapeutic efficacy without toxicity.
- Mechanism(s)?? – Important
- Preclinical models will play key role in evaluating toxicity of new combinations.

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NSCLC Autophagosome Cancer Vaccine

- Human NSCLC cell lines (cGMP)
  - adenocarcinoma
  - mixed histology (Squamous/adeno)
- Eight NCI prioritized cancer antigens
- Agonists for TLR 2, 3, 4, 7 and 9
- Reproducibility across 6 lots (Gene/Protein)
- Stability out 23 months

UbiVac

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Clinical Trial:

Phase II Trial of Cyclophosphamide with Allogeneic Non-small Cell Lung Cancer (NSCLC) Dribble Vaccine alone or with Granulocyte-Macrophage Colony-Stimulating Factor or Imiquimod for Adjuvant Treatment of Definitively-Treated Stage IIIA or IIIB NSCLC

UbiVac

Earle A. Chiles Research Institute

Earle A. Chiles Res. Inst.

Shawn Jensen
Sachin Puri
Tarsem Moudgil
Chris Twitty
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Michael LaCelle
Christopher Paustian
Michael Afentoulis
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Christopher Dubay
David Messenheimer
Tyler Hulett

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Rachel Sanborn
Carlo Bifulco

CBER / FDA
Raj Puri
Barhat Joshi
Bhaskar Bhattacharya
Jing Han
Amy Yang

UbiVac
Sandra Aung
Traci Hilton

Mayo Clinic
Helen J. Ross

NCI
R01 CA119123 (Fox)
R21 CA123864 (Urba)
R43 CA121612 (Aung)
R44 CA121612 (Aung)

American Cancer Society with funding from the New England Division SpinOdyssey

Safeway Foundation

Providence Med Found
Chiles Foundation
Robert Franz
Wes & Nancy Lematta

Why not use NHP for evaluating vaccine toxicity?

Cynomolgus macaques are a standard species used preclinically for evaluating efficacy and toxicity of therapeutic drug candidates and vaccines. However, findings from preclinical studies conducted in cynomolgus macaques are often highly variable, which may in part be attributed to genetic diversity.

Wu H, Adkins K. Mamm Genome. 2012 Aug;23(7-8):467-77

Why not use NHP for evaluating vaccine toxicity?

- Genetic polymorphisms were identified in 49 genes of the immune system using DNA isolated from 40 cynomolgus macaques.
- 20 originated from a breeding center in China and 20 were of Mauritian origin.
- A total of 580 polymorphisms were identified, including 561 SNPs, 9 deletions, and 10 insertions
- Genes of the immune system in cynomolgus macaques are highly polymorphic.

Wu H, Adkins K. Mamm Genome. 2012 Aug;23(7-8):467-77