Chemical Biology Consortium: LDHA

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LUDWIG CANCER RESEARCH

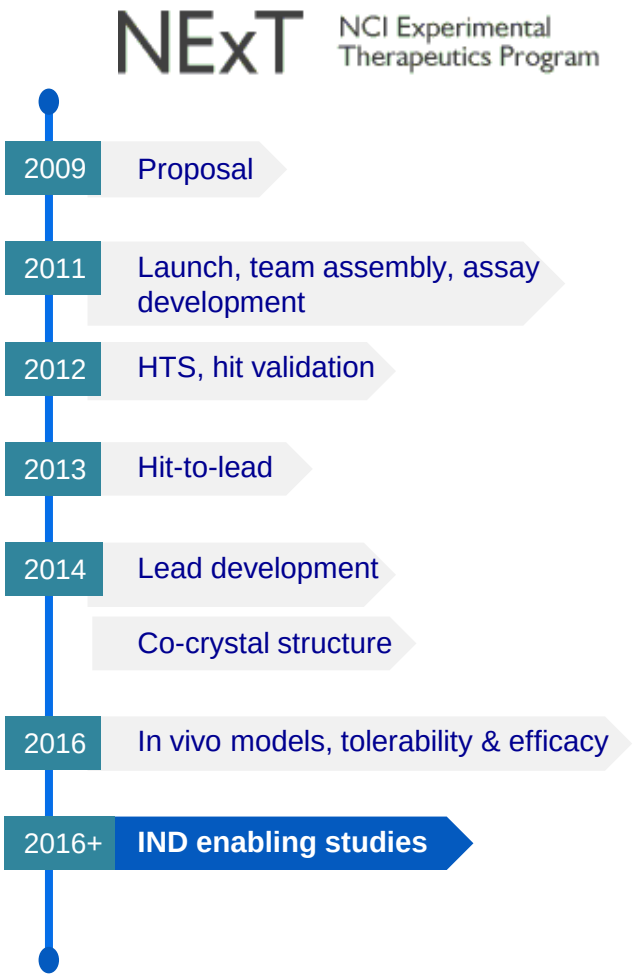
THE WISTAR INSTITUTE

Target: Lactate Dehydrogenase A/B

Team:

Applicant (PI): *Chi Dang, U Penn*
NCI Project Leader: *Len Neckers, NCI CCR*
PIs & Centers: *Matt Hall, NCATS*
Alex Waterson, Vanderbilt
Myrtle Davis, Melinda Hollingshead, NCI DTP
Victor Darley-Usmar, UAB
Larry Sklar, Jeff Norenberg, UNM
Scientific Project Manager (SPM):
Gordon Stott, Leidos Biomed
Project Manager (PM): *Dane Liston, NCI*

Project Objective: Discovery and Development of a Lactate Dehydrogenase A (LDHA) Inhibitor as an Antineoplastic Agent

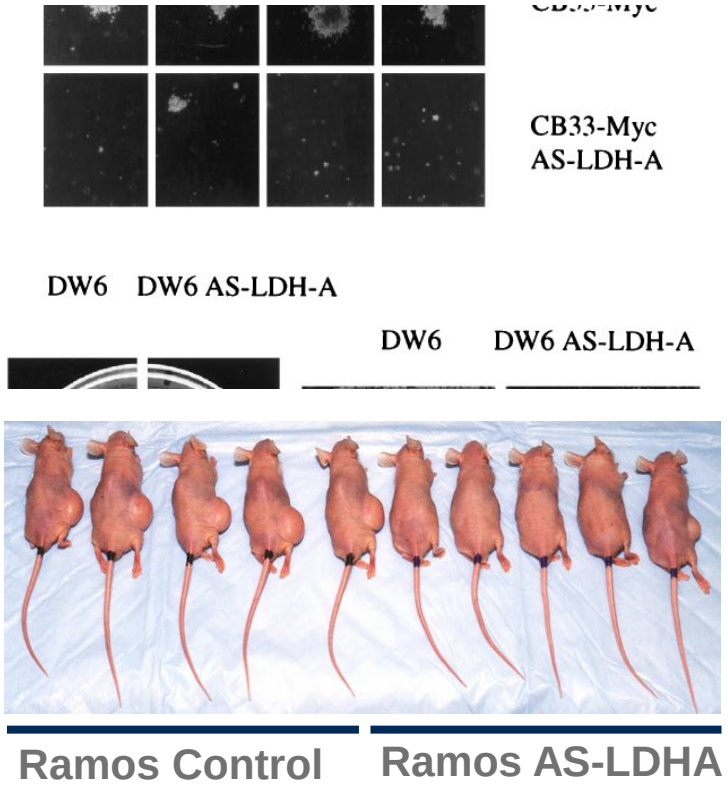


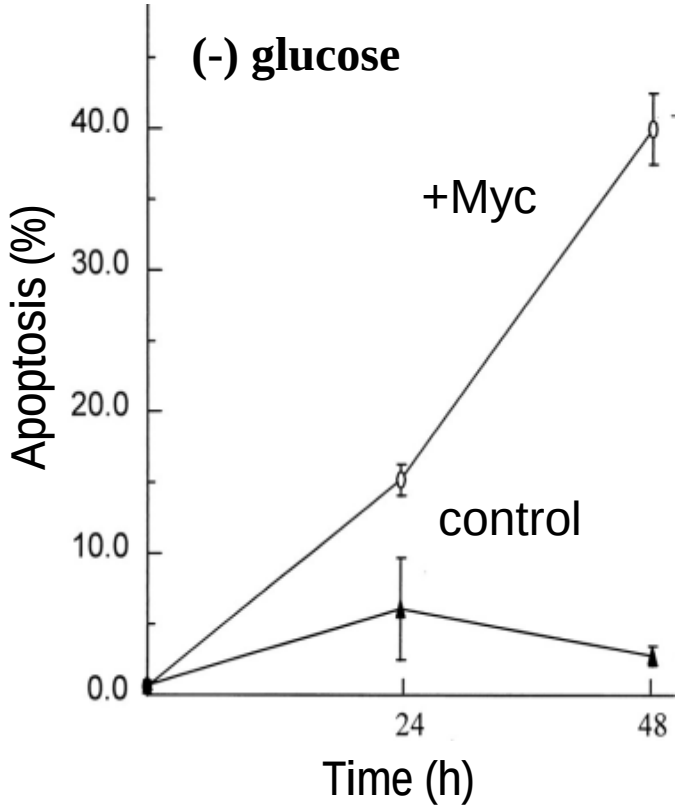
Myc and LDHA

c-Myc transactivation of *LDH-A*: Implications for tumor metabolism and growth

(oncogene/lactate dehydrogenase/hypoxia/tumorigenicity)

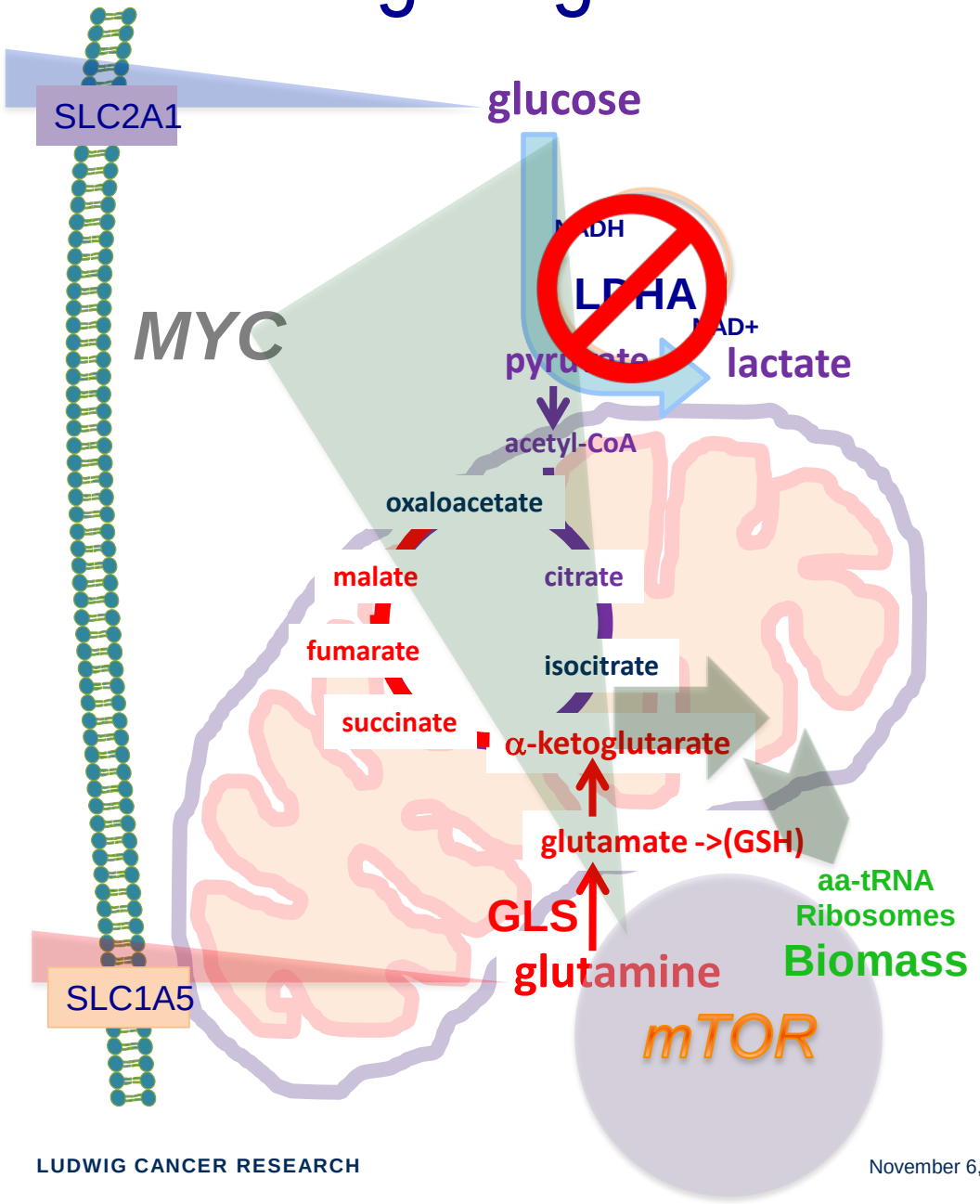
HYUNSUK SHIM*, CHRISTINE DOLDE†, BRIAN C. LEWIS†, CHYI-SUN WU*, GERARD DANG*, RICHARD A. JUNGMANN‡, RICCARDO DALLA-FAVERA§, AND CHI V. DANG*†¶‖**



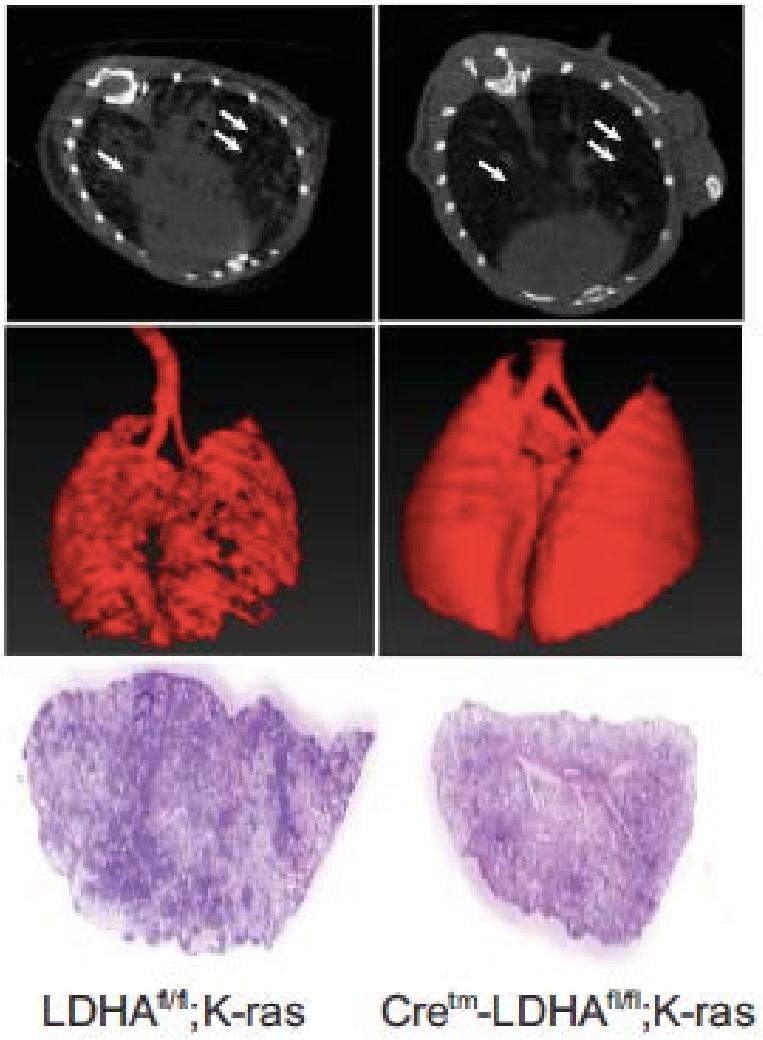


Shim H et al. PNAS 1998;95:1511-1516

Targeting Tumor Energy Pathways



Genetic evidence: LDHA
Xie et al. Cell Metab 2014



Targeting Tumor Energy Pathways

1920's

1997

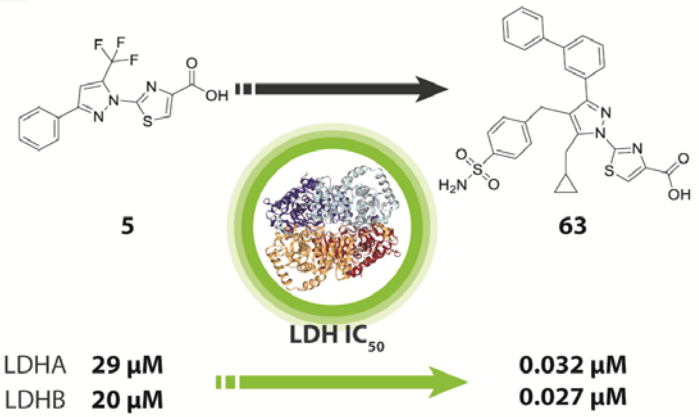
MYC regulates LDHA – required for lymphoma cell growth
Shim et al., PNAS 1997

NExT NCI Experimental Therapeutics Program

Chemical Biology Consortium

2018

J Med Chem, in press



LDHA Project

Clinical Opportunities: .

- Tumors which exhibit *high glycolytic flux*.
- Tumor microenvironments which are *hypoxic*.
- Tumors with inherent *defects in mitochondrial* oxidative phosphorylation.

Challenges:

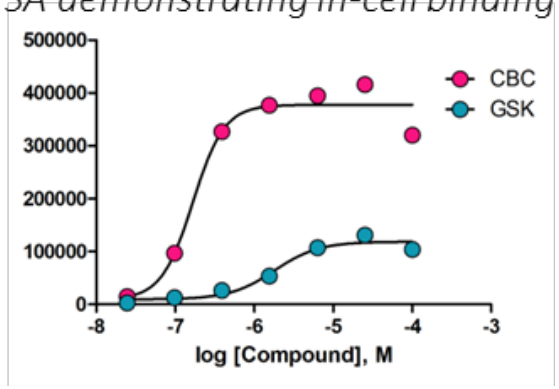
- High cellular concentrations of LDHA/B
- Constraints on pharmacokinetics for extended periods of high level target engagement
- Metabolic plasticity is a path to resistance.

Meeting The Challenge

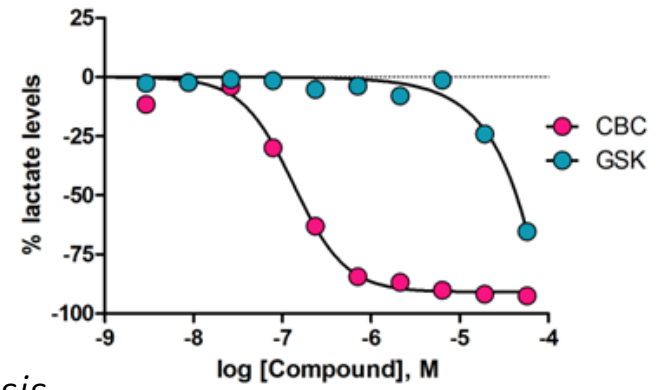
High cellular concentrations of LDH:

- Lead series includes 2 specific and potent orally bioavailable inhibitors of LDH.
- High target affinity and long target engagement times by SPR and CETSA.
- In tumors >80% of the enzyme for 3 hours or more through IV dosing (006 and 737).
- Ability to suppress glycolysis both *in vitro* and *in vivo*

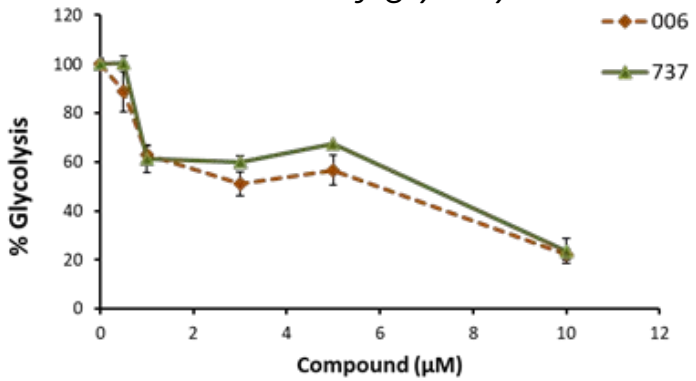
CETSA demonstrating in-cell binding to LDHA



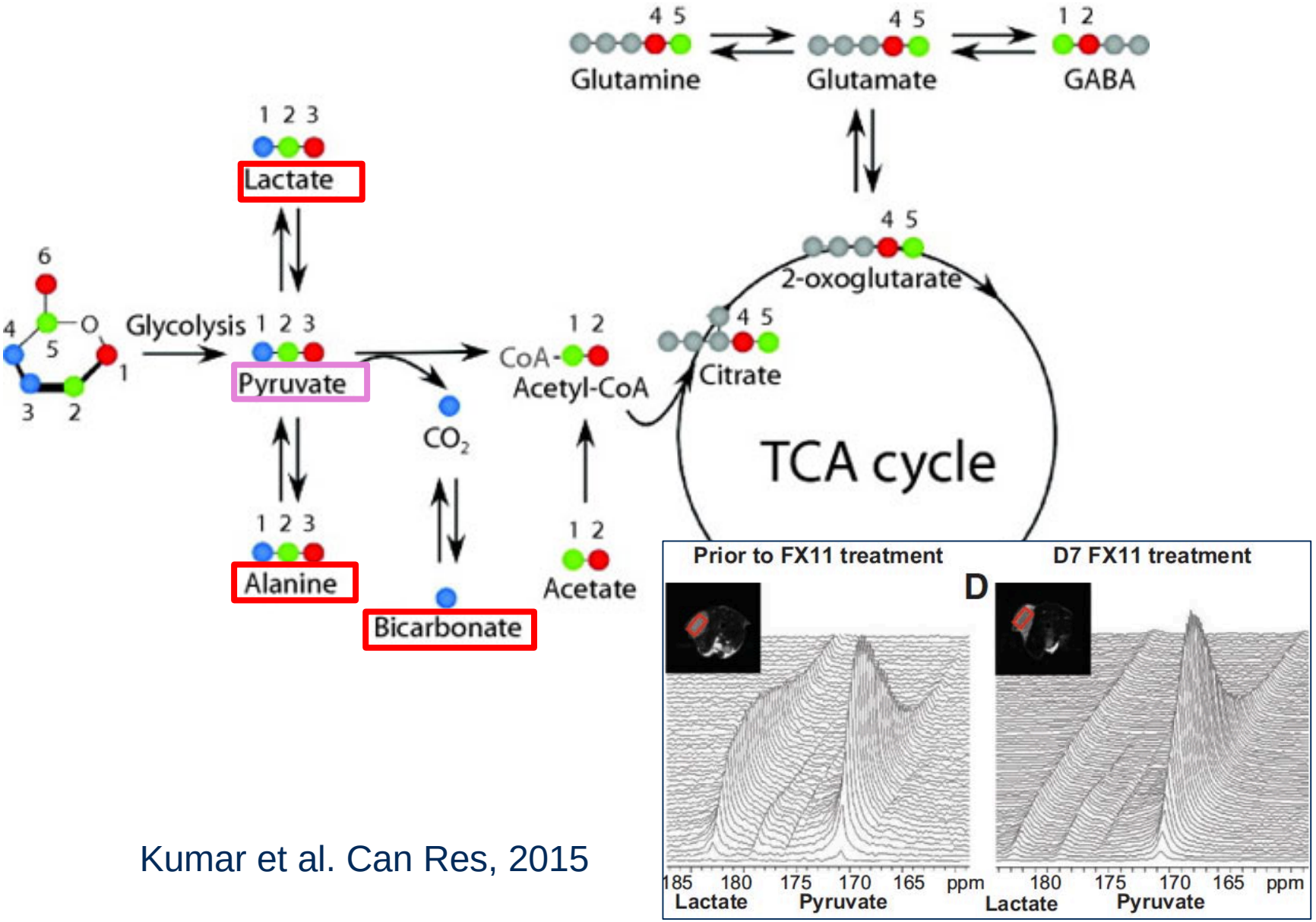
Cellular lactate inhibition



Inhibition of glycolysis



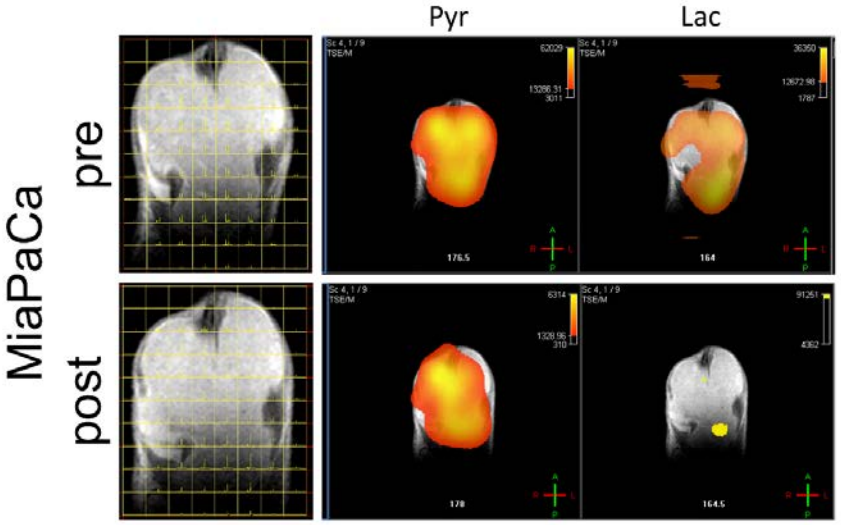
In vivo hyperpolarized ^{13}C-pyruvate MRS

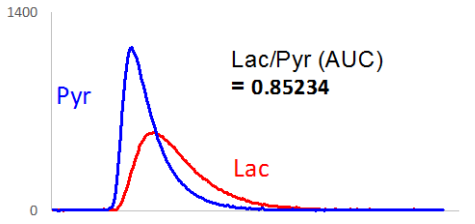


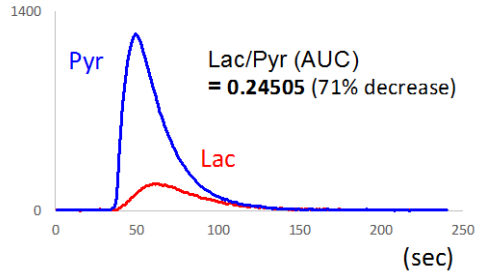
Kumar et al. Can Res, 2015

Meeting The Challenge

Demonstrating Target Engagement in vivo with direct Pyruvate to Lactate flux measurements

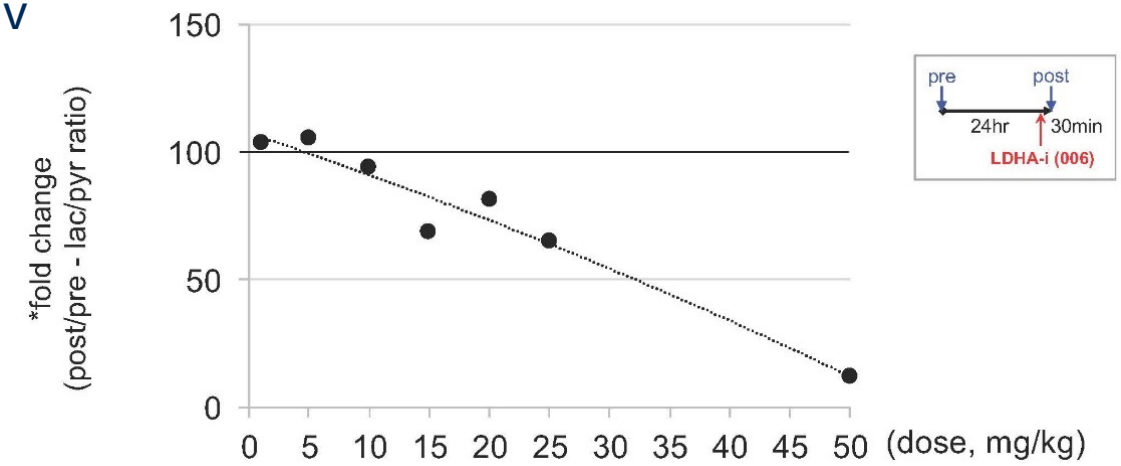




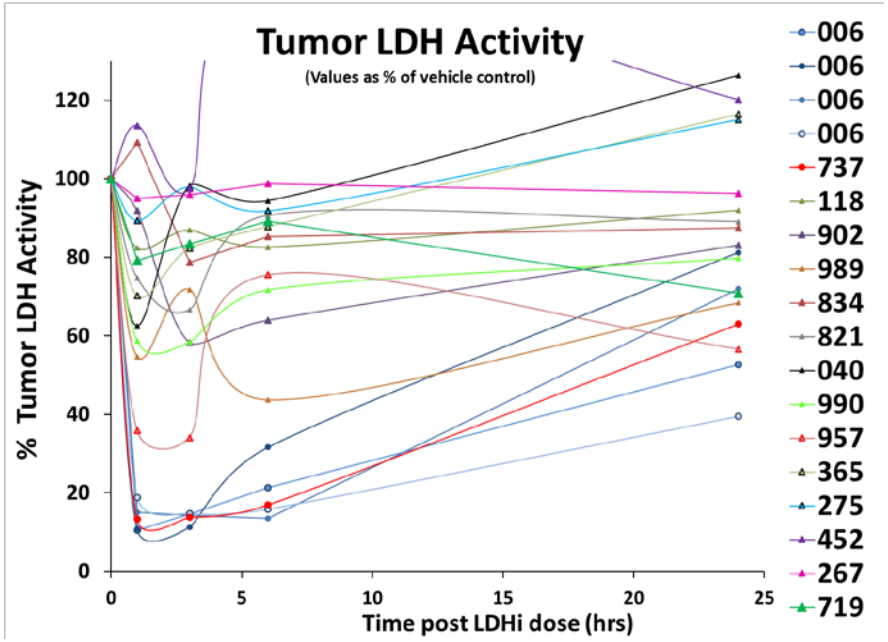


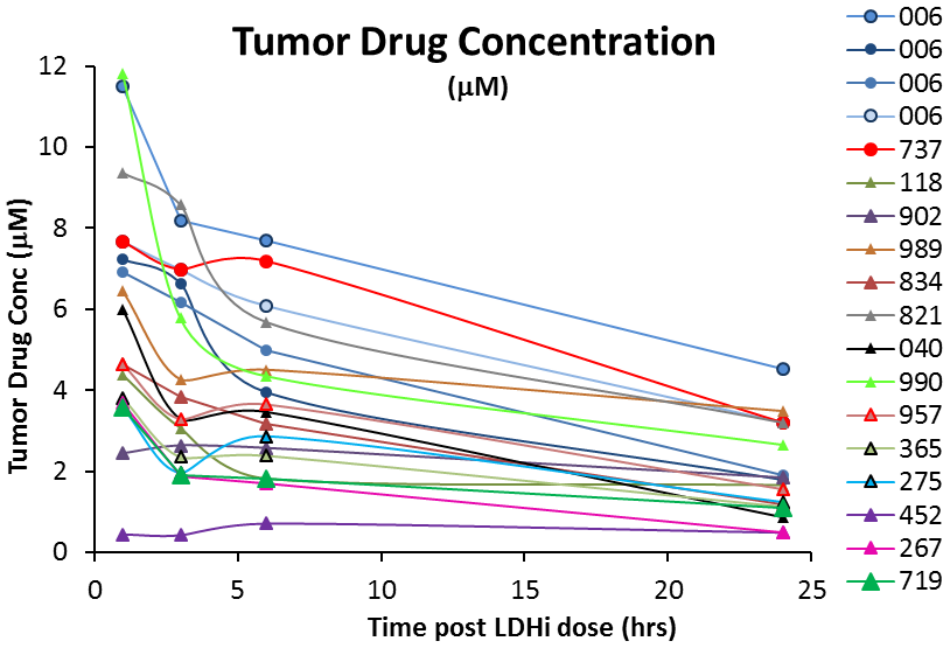
Post = 30 min post single i.v dose of 50 mg/kg 006

LDH inhibition, dose response



Demonstrating Target Engagement in vivo

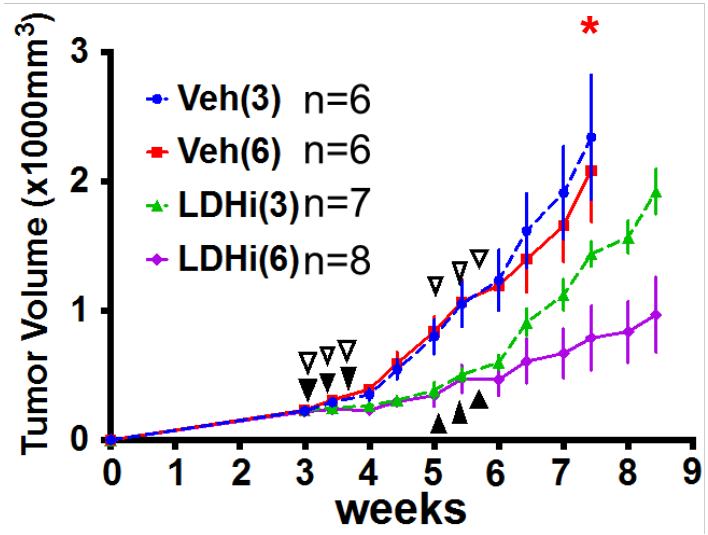


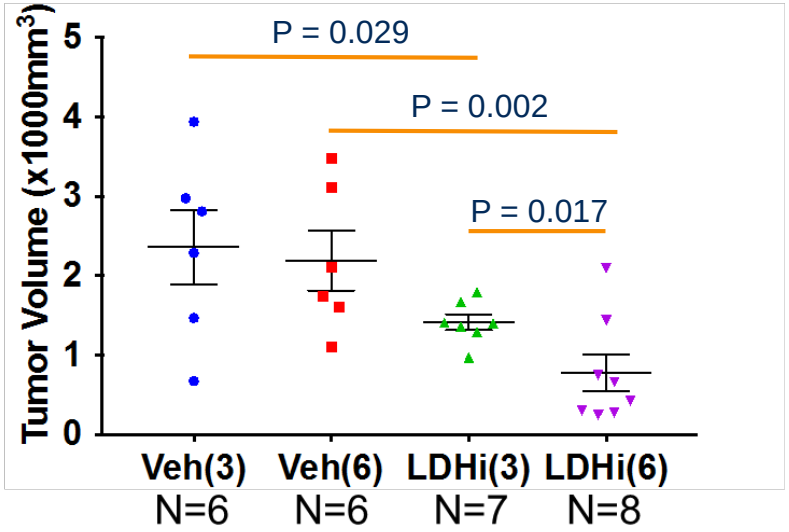


- The team has screened 15 LDH inhibitors in A673 xenografts through IV dosing.
- LDHA inhibition: durabie response correlates with tumor drug levels.
- The pattern of LDH inhibition matches well with MRS imaging studies.
- Similar levels of LDH inhibition are also seen in MiaPaCa-2 and HL60 tumor xenografts dosed IV with 006

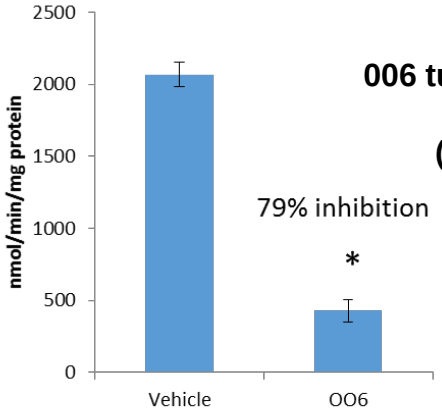
Meeting The Challenge

Tumor Growth Suppression in vivo in MiaPaCa Xenografts





Lactate Dehydrogenase



T-test 0.000051

006 tumor concentration:
12.9 ± 1.4 µM
(m ± s.e.m. n=4)

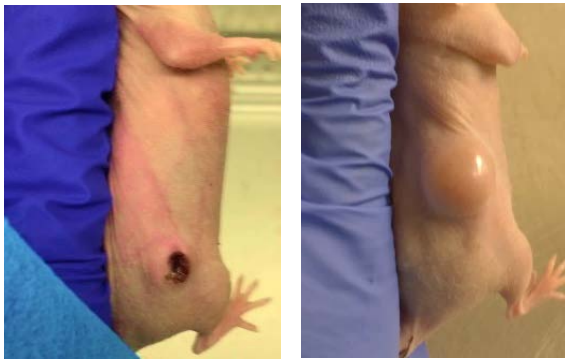
006 dosed IV at 50 mg/Kg q2d x 3;
tumors harvested @ 1 hr post last dose

Meeting The Challenge

Tumor Growth Suppression in vivo in MYC-inducible lymphoma Xenografts

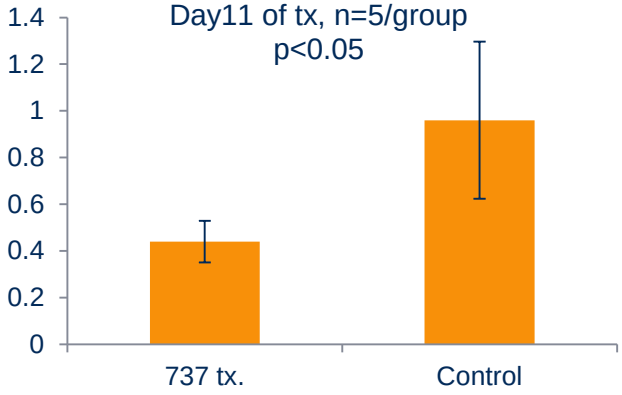
P493 tumor

Xiang et al., unpublished

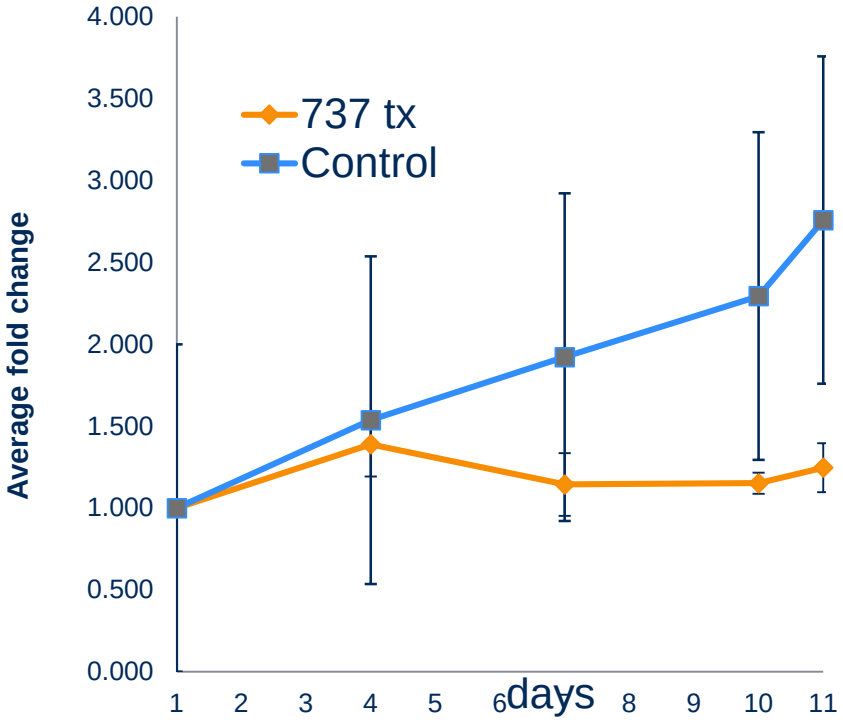


737 Rx control

Tumor Weight(gram)

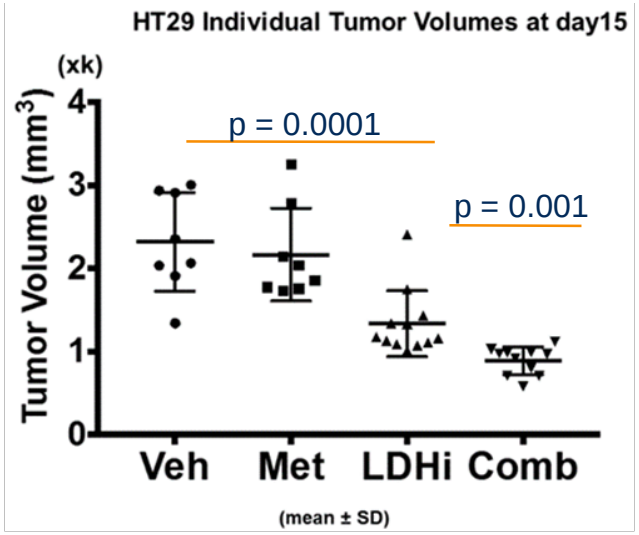


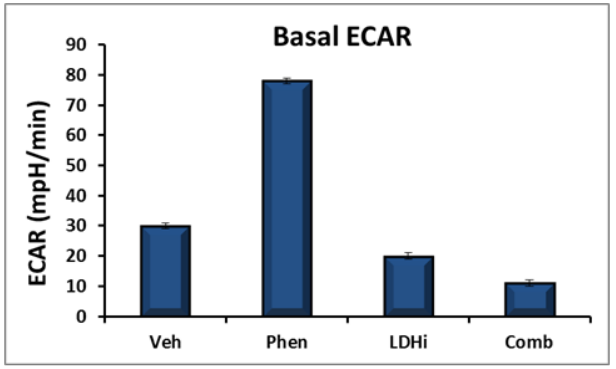
737 50mg/kg PO qd
x 11days, n=5; p<0.05
Initial tumor sizes ~500mm^3



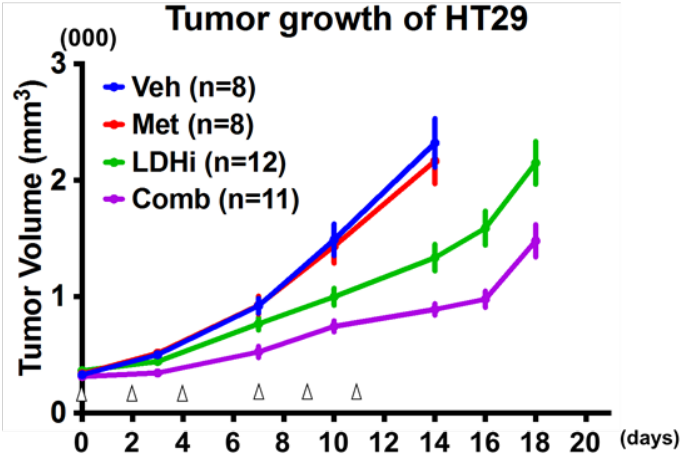
Meeting The Challenge

Combination therapies may lead to increased efficacy in vivo

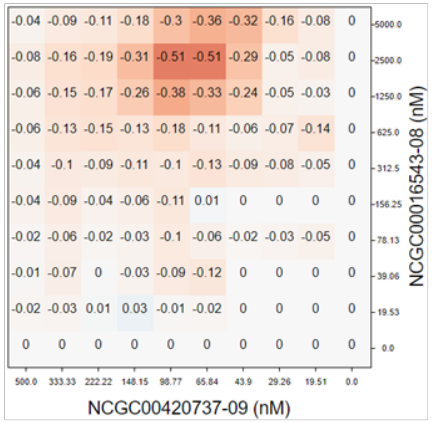




Increasing glycolytic flux with Phenformin increases the sensitivity of A673 cells to LDH inhibition

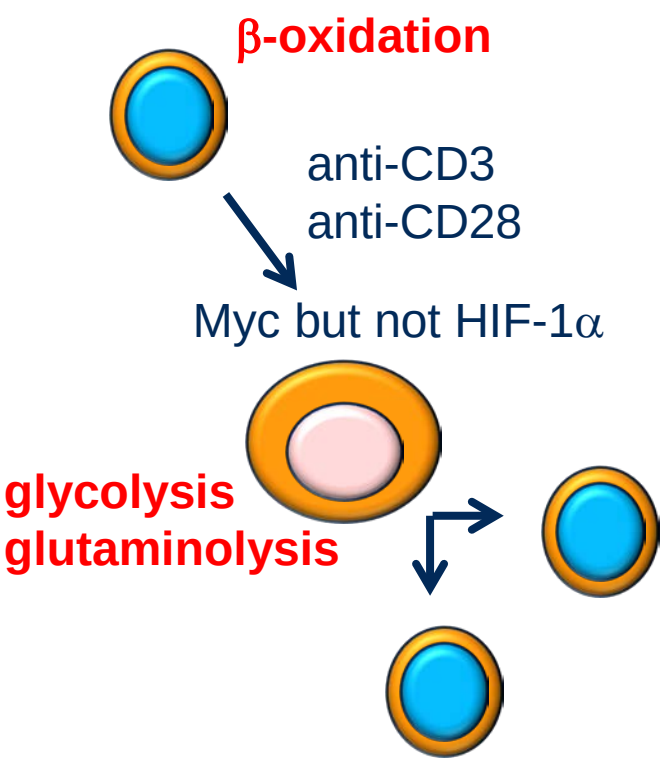


Tumor cells were inoculated in leg. IV administration commenced on day 0 and 30 mg/kg 006 or vehicle was administered through tail vein injection for two cycles separated by one week (1 cycle = M/W/F). Metformin given as 50 mg/kg PO, 006 administered 2h after Metformin.

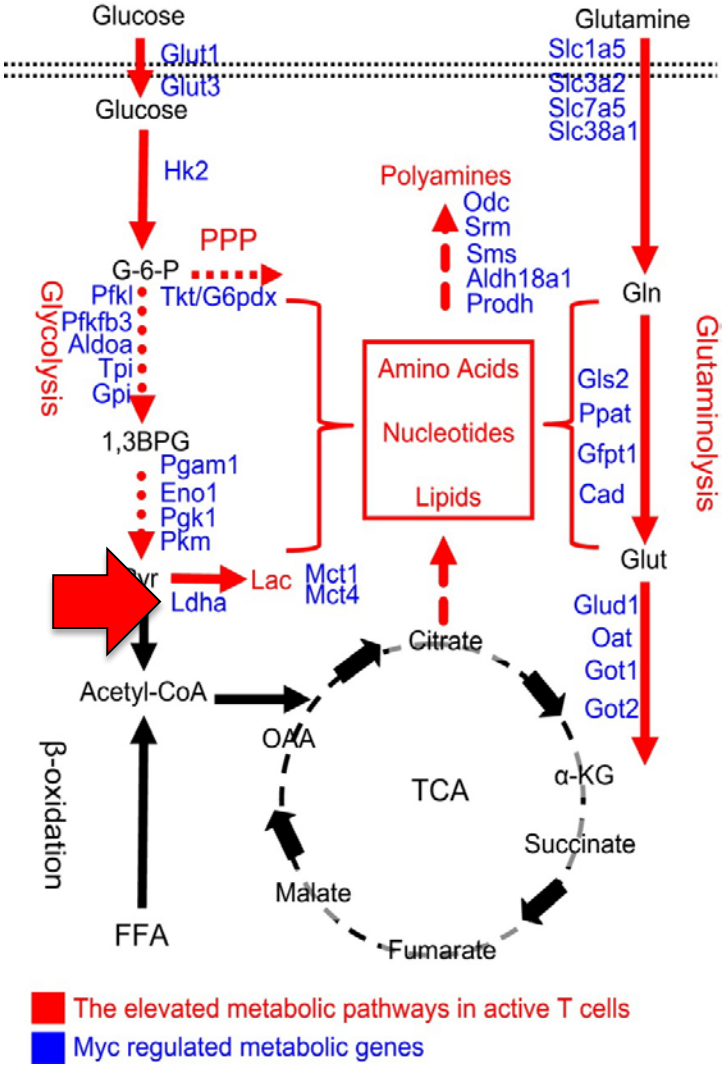


Mitochondrial active compounds show strongest synergy with LDH inhibitors

Bioenergetics of activated T cells: Myc & cell growth

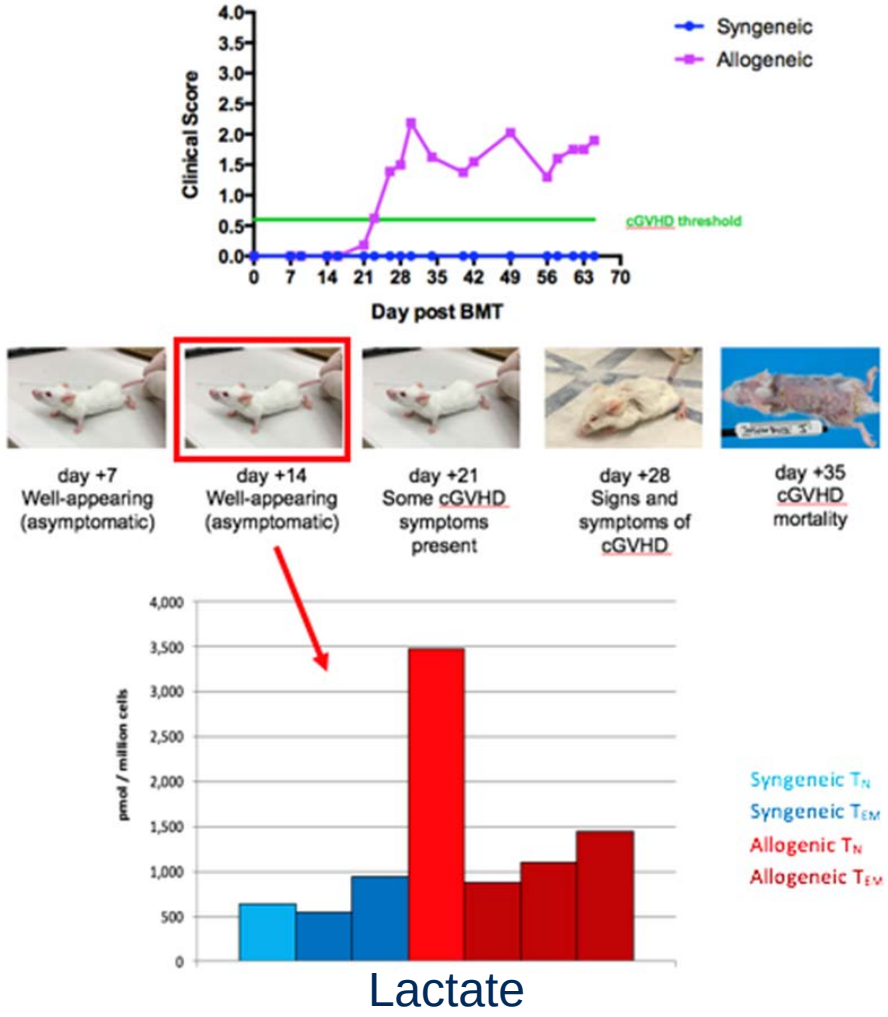


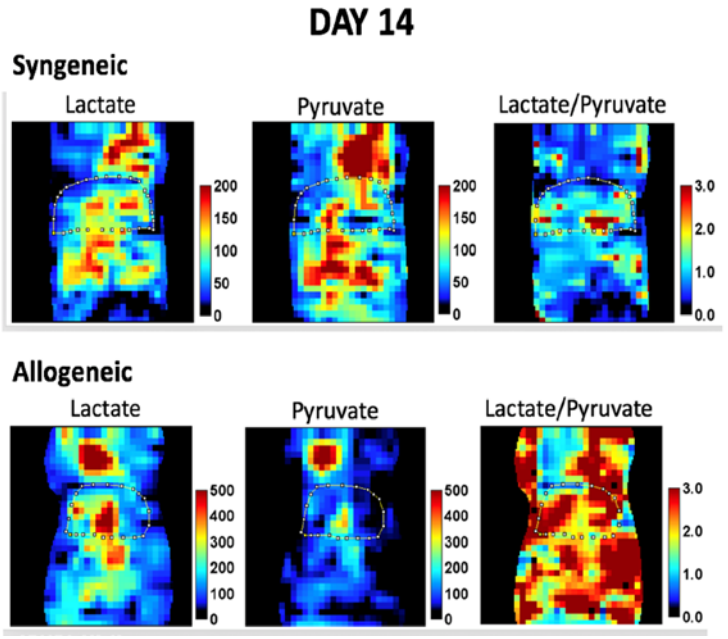
Wang et al., Immunity 2011



Meeting The Challenge

Graft vs Host Disease: Targeting a metabolic switch in vivo



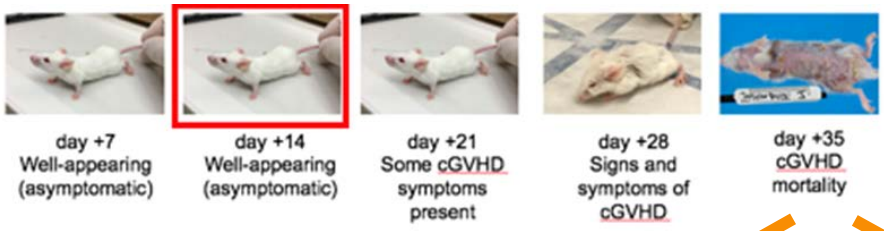


- Hyperpolarized 13C-pyruvate *in vivo* NMR/MRI conducted on syngeneic and allogeneic recipients at post HSCT day +14:
- The lactate : pyruvate ratio is increased in the allogeneic graft recipients before they have clinically-evident cGVHD

** In collaboration with Gress lab @ NCI

Meeting The Challenge

Graft vs Host Disease: Targeting a metabolic switch in vivo



Treatment: d7 – d14

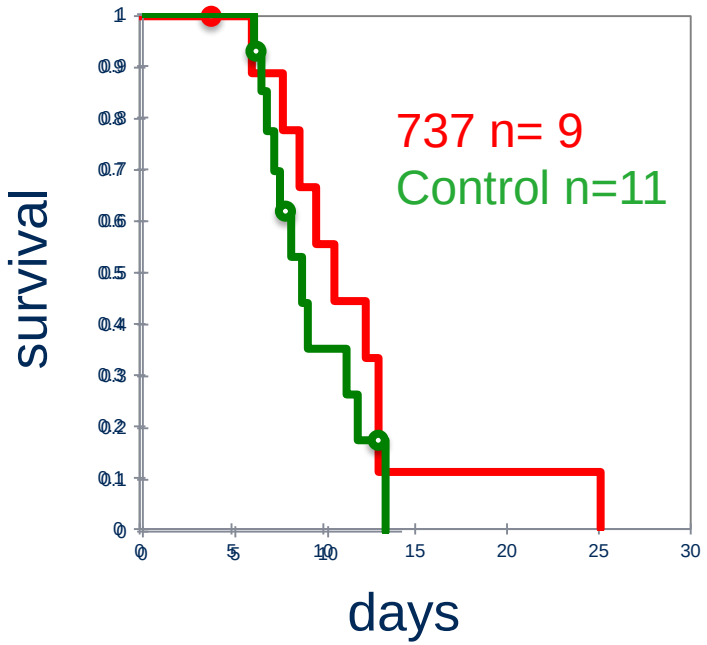
Treatment: d14 – d21

Day 40

Daily treatment by **oral** gavage with 006 (25 mg/kg)

Summary of *in vivo* Findings

- Anti-tumor efficacy appears to require sustained (2–6 hours) target inhibition of greater than 60%
- Identified potentially effective combination therapy in preclinical *in vitro* and *in vivo* models
- Identified additional therapeutic indications based upon mechanism of action: GVHD
- Effects on immunity: therapeutic window and indications



MYC-inducible
GEMM
Liver Cancer

Xiang, unpublished:
No evidence of tumor acceleration in immunocompetent mice

Meeting The Challenge

Repeating and extending the initial efficacy findings

cGVHD Model – Ron Gress (NCI)

- Build on successful pilot study with limited **oral** dosing of LDH inhibitors

MiaPaCa Model– Melinda Hollingshead (NCI)

- Repeat MiaPaCa experiment with both oral and IV dosing
- Replicate differential sensitivity of leg vs flank xenografts to IV 006

Ewing's Sarcoma model - Christine Heske (NCI)

- Evaluate impact of LDHi on regrowth of sarcoma xenografts following irinotecan therapy; prevention or delay by LDH inhibition is of significant clinical relevance

Meeting The Challenge

Moving away from IV dosing towards models suitable for oral or local administration

HCC model – Brad Wood (Interventional Radiology, Clinical Center)

- Leverage high and persistent liver drug concentrations achieved after **oral** dosing

GBM model –Kevin Camphausen (NCI)

- Drug delivery by in-dwelling port bypasses systemic circulation and is highly clinically relevant

Disseminated liquid tumor model (UNM)

- High plasma drug concentrations achieved by **oral** dosing of LDH inhibitors

Goals from the ***Previous*** Twelve Month Period

- Complete Tier 2 and 3 ADME assessments of lead compounds
- Identify one or more sensitive *in vivo* models in addition to P493
- Evaluate the efficacy and tolerability of additional LDHA inhibitors with alternate PK profiles *in vivo*
- Identify the most effective dosing schedule
- Investigate potentially synergistic combination therapies
- Provide insight into mechanism(s) of anti-tumor activity

LDHA Project Goals for next 12 months

- Explore other routes and in vivo models through collaborations
- Assess disseminated blood cancer model
- Perform tolerability and toxicology studies to establish a therapeutic index
- Optimize synergistic combination therapies
- Continue exploration of utility for cGVHD
- Nominate candidate for clinical development

Questions?