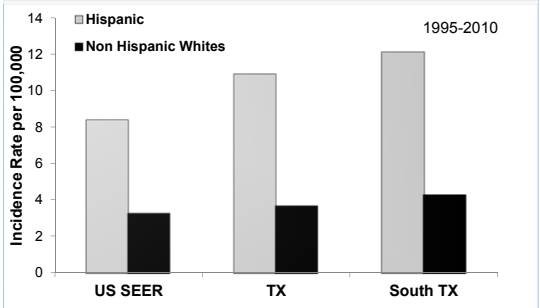
Improving Therapy for Hepatocellular Carcinoma by Combining a HDAC Inhibitor with an Alkylating Agent

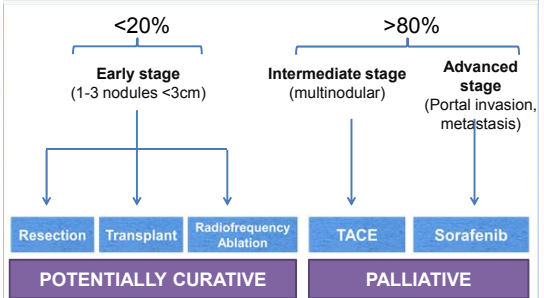
Jessica Zavadil
CPRIT Innovations in Cancer Prevention and Research
9/10/2015

High Incidence of Liver Cancer in South Texas Latinos



PLoS One (2014) 9(6):e99365

HCC Diagnosis and Therapy

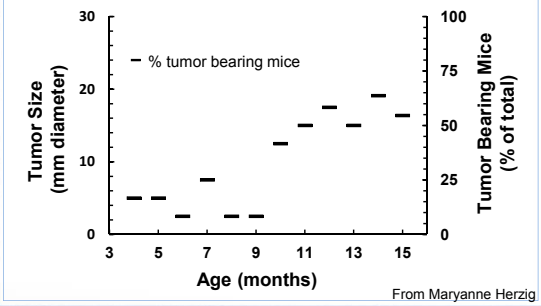


Modified from Hepatology (2011), 53(3):1020-2

C3HeB/FeJ HCC Has Tumor Characteristics Similar to Human HCC

Tumor Characteristic	Human HCC	C3HeB/FeJ HCC
More prevalent in males	++	+++
Occurs later in life	++	++
Variable size	++	++
Variable location in liver	++	++
Multiple foci	++	++
Hepatosteatosis	++	++
Cirrhosis/fibrosis	+++	+
Progression from dysplasia to vascular invasion	++	++
Biomakers (p53, GP3, HSP70, AFP, TGFβ1)	++	++
No single gene drives majority of tumors	++	++

Tumor Prevalence and Size in C3HeB/FeJ Males Increase with Age



From Maryanne Herzig

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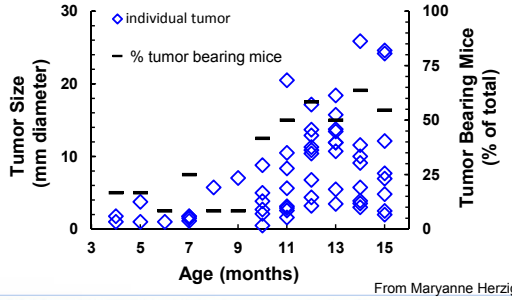

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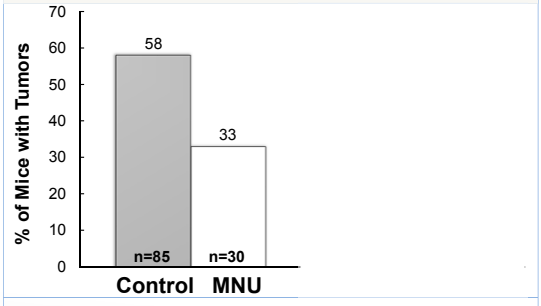
Monofunctional Alkylating Agents Reduce Tumor Prevalence in C3HeB/FeJ Males

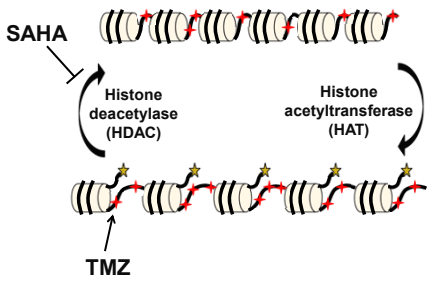


Temozolomide (TMZ) as an Anti-cancer Agent

- First line therapy for glioblastoma
- O^6-methylguanine DNA methyltransferase (MGMT) expression affects susceptibility
  - TMZ induces lesions which are repaired by MGMT
  - Highly mutagenic and cytotoxic if unrepaired
  - Overexpression is associated with therapy resistance
- MGMT is silenced in 40% of human HCC through promoter hypermethylation

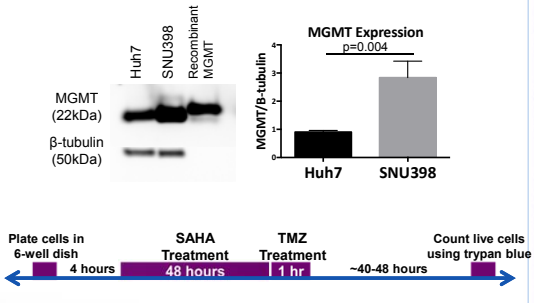
Int J Cancer (2003) 103:440–444

Proposed Use of SAHA to Maximize TMZ Efficacy

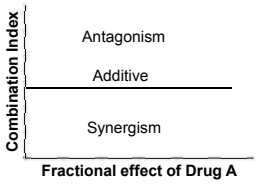


Do SAHA and TMZ synergistically reduce cell viability *in vitro*?

Experimental Design for Treatment of HCC Cell Lines with SAHA and TMZ



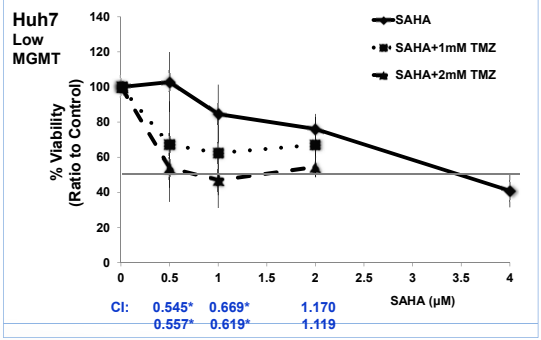
Chou Talalay Statistical Test for Synergy



Combination Index (CI):
- <0.1 very strong synergism
- 0.1-0.3 strong synergism
- 0.3-0.85 synergism
- 0.85-1.45 additive
- 1.45-3.33 antagonism

Compusyn User's Guide (2005)

Synergistic Reduction in Cell Viability in Both Cell Lines



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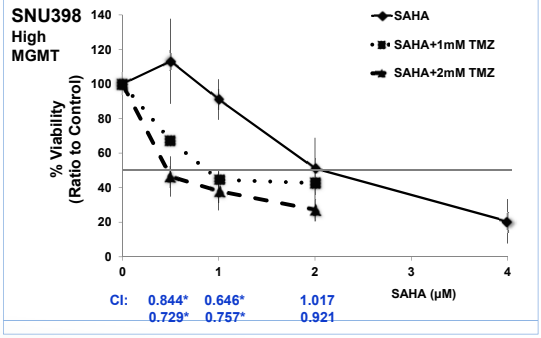
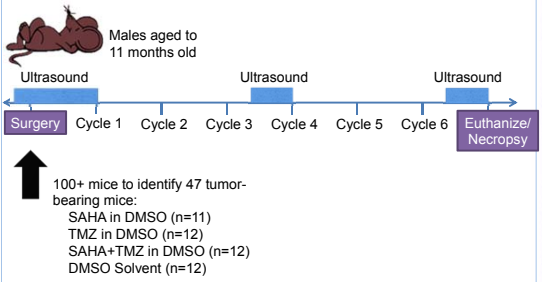


Do SAHA and TMZ synergistically reduce tumor burden *in vivo*?

Test the hypothesis that tumors will be responsive to the combination of SAHA and TMZ



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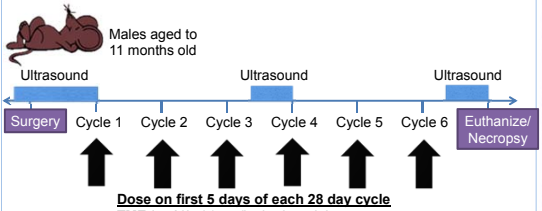
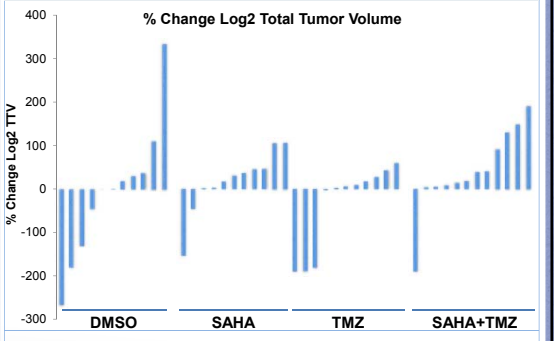
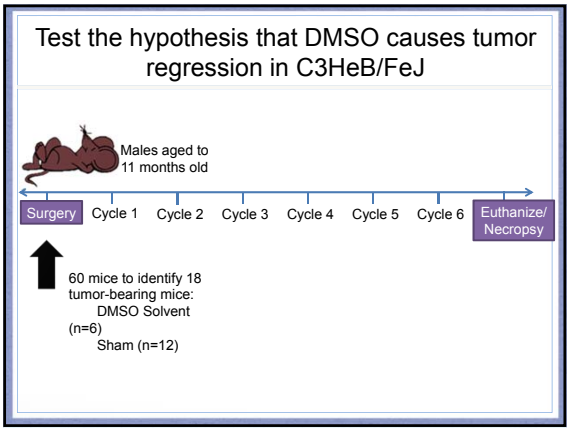


Dose on first 5 days of each 28 day cycle
TMZ (n=12): 31 mg/kg body weight
SAHA (n=11): 100 mg/kg
SAHA+TMZ (n=12): 85 mg/kg SAHA
22 mg/kg TMZ
DMSO (n=12): solvent control

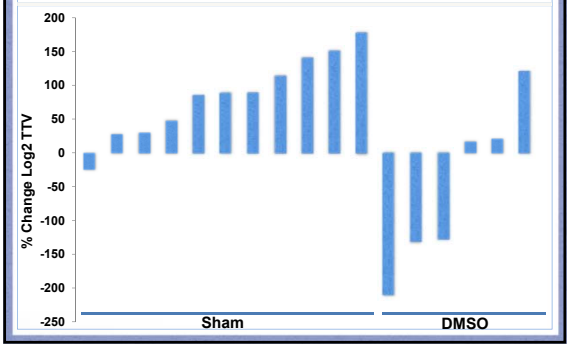
All Groups Have Variability in Tumor Response



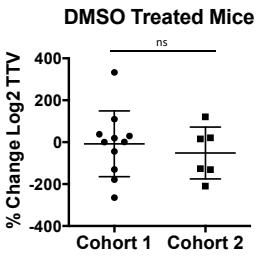
Test the hypothesis that DMSO causes tumor regression in C3HeB/FeJ



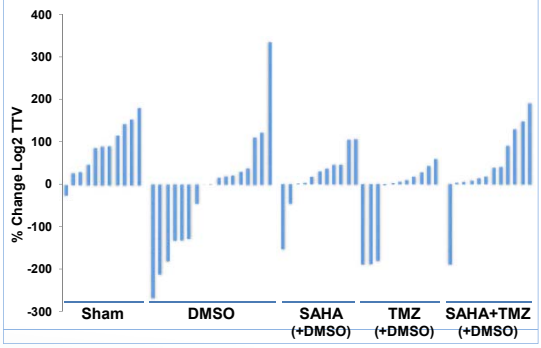
DMSO Causes Tumor Regression at Sublethal Doses



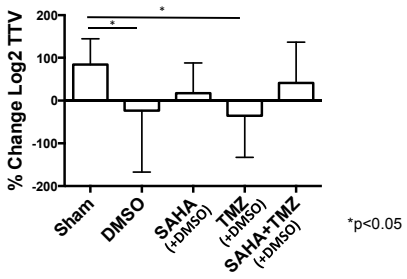
A Similar Response to DMSO Was Seen in Both Experiments



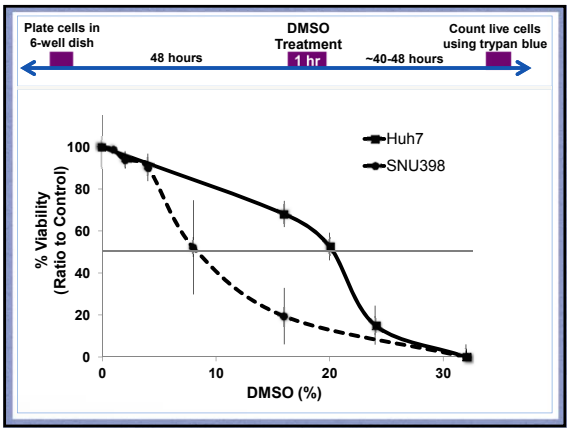
Combined Data from *In Vivo* Drug Treatment Studies



Significant Reduction of Tumor Burden with TMZ or DMSO Treatment



*p<0.05

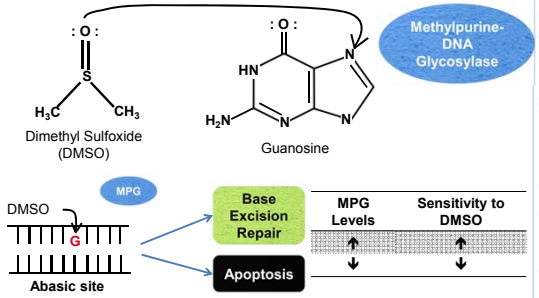


DMSO: Innocuous?

- Considered non-toxic by EPA
- Frequently used as a solvent for chemicals with poor solubility and as a cryopreservative in the laboratory
- FDA approved as a cryopreservative for bone marrow transplants, to treat cystitis, and off label use for extravasation side effect of chemotherapy infusions
- 10% DMSO is synergistic with several chemotherapies in ovarian cancer primary cells

Ann Oncol (2004) 25(6):858-62
Am J of Obstet Gynecol (1988) 159(4): 848-52

Hypothetical Model of DMSO Action in HCC



Conclusions To Date

1. SAHA and TMZ synergistically reduce viability of 2 HCC cell lines *in vitro*.
2. TMZ (in DMSO) and DMSO significantly reduce total tumor burden in C3HeB/FeJ HCC, but some tumors do not respond to treatment.
3. SNU398 is more sensitive to both DMSO and TMZ compared to Huh7.

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