A New Pharmacological Approach In Brain Metastases

5th Annual Brain Metastases Research And Emerging Therapy Conference

Frits Thorsen

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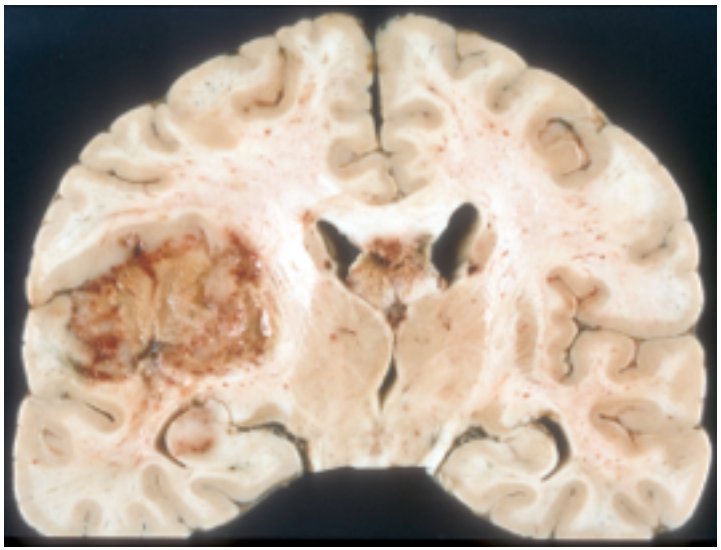
MIC
Molecular Imaging Center

Primary versus secondary malignant brain tumors

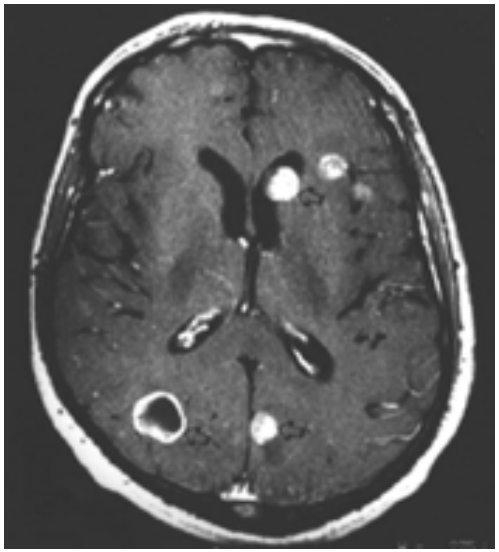
Primary malignant brain tumors



Estimated ≈ 24 600 new cases in the US in 2013.
Gliomas ≈ 80% of all malignant primary brain tumors.
GBMs ≈ 54% of all gliomas (≈10 600/yr in the US).
Median survival < one year.

http://www.abta.org/news/brain-tumor-fact-sheets/

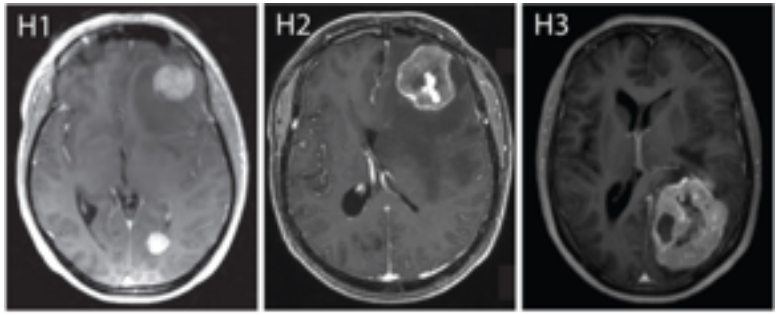
Brain metastases

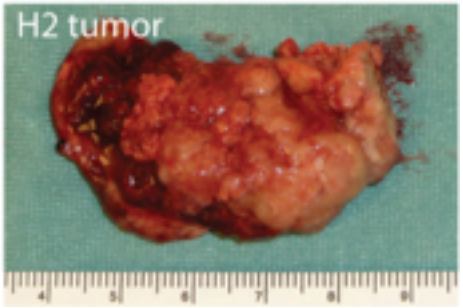


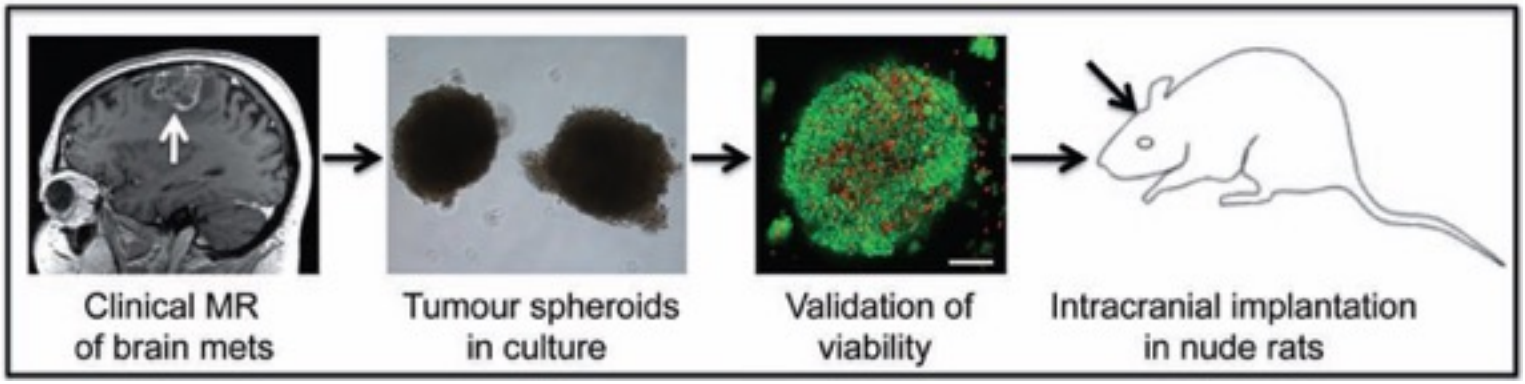
From cancers in lung (50%), breast (20%), skin (10%).
170 000 new patients/year in the US.
Occur in around 30% of all cancer patients.
2/3 of the patients have multiple brain metastasis.
Median survival ranges from 2.3 to 13.5 months.
Increasing incidence.

Daphu I, …, Thorsen F, Clin & Exp Metastasis 2013
Norden A et al, Curr Opin Neurol 2005

Brain metastasis growth model in nude rats





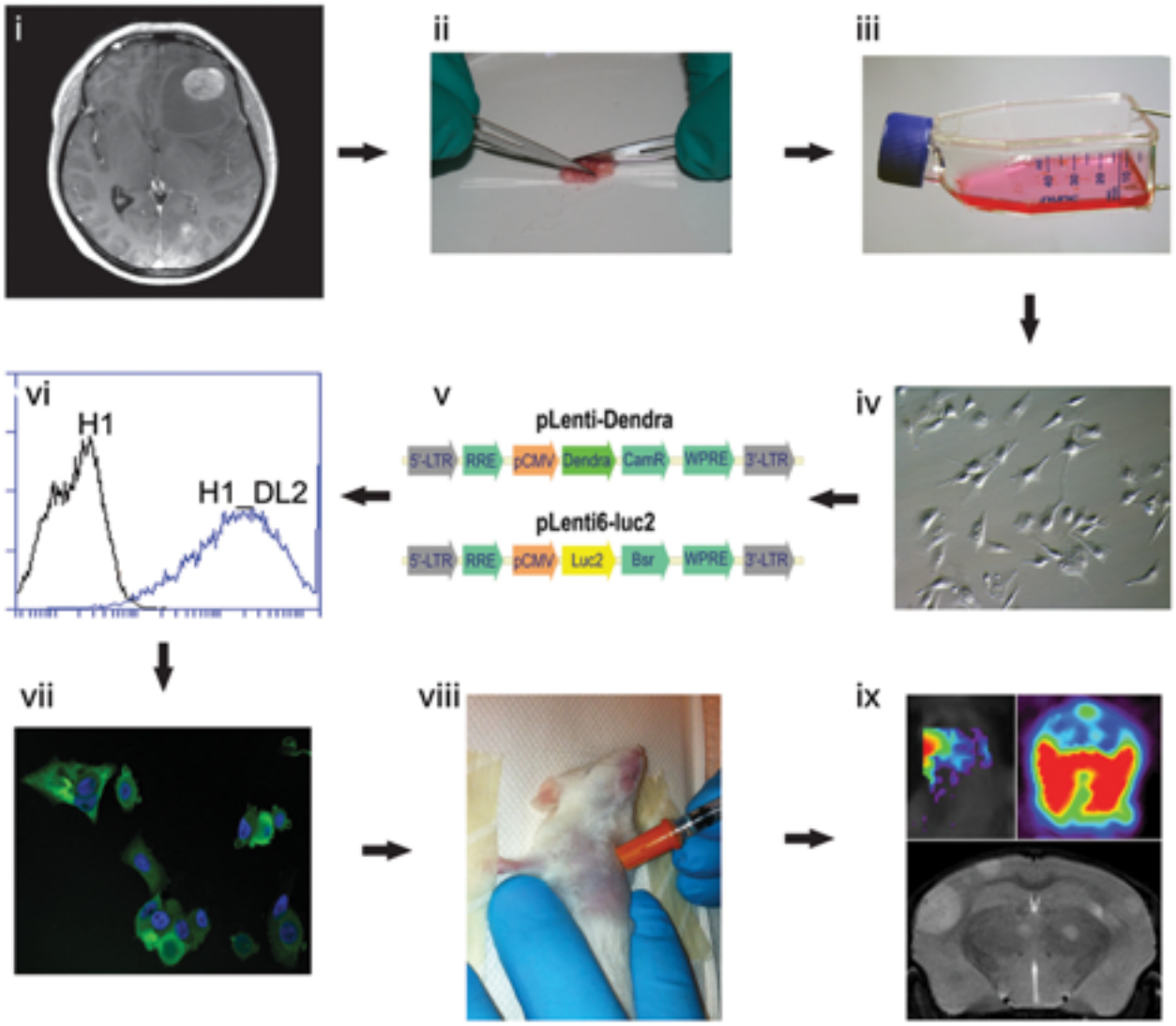


Neuropathology and Applied Neurobiology (2011), **37**, 189–205 doi: 10.1111/j.1365-2990.2010.01119.x

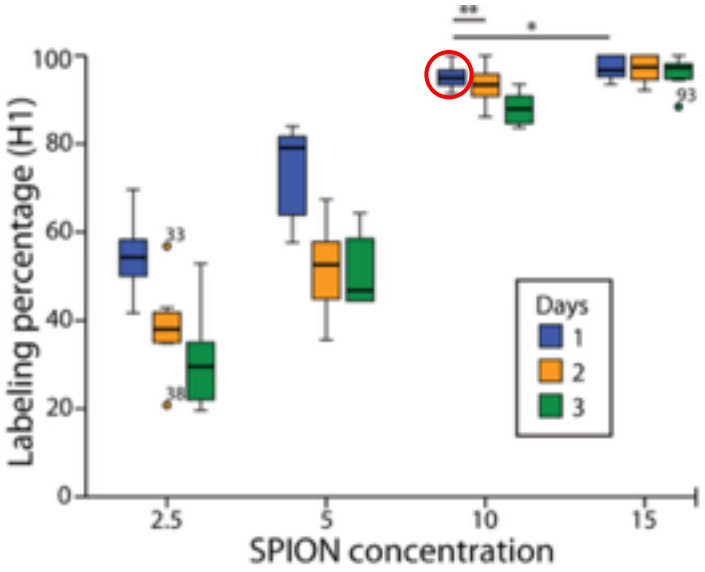
A novel brain metastases model developed in immunodeficient rats closely mimics the growth of metastatic brain tumours in patients

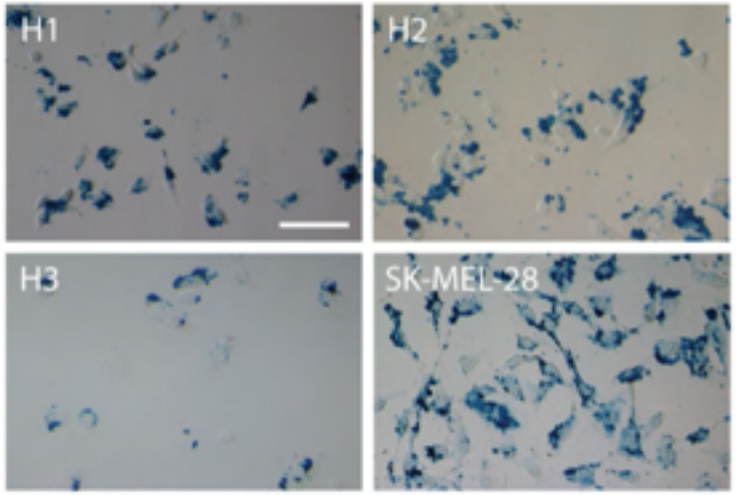
J. Wang*††, I. Daphu*††, P.-H. Pedersen†, H. Miletic*, R. Hovland‡, S. Mørk§, R. Bjerkvig*¶, C. Tiron*, E. McCormack**, D. Micklem*, J. B. Lorens*, H. Immervoll§ and F. Thorsen*

Ectopic brain metastasis model in NOD/SCID mice

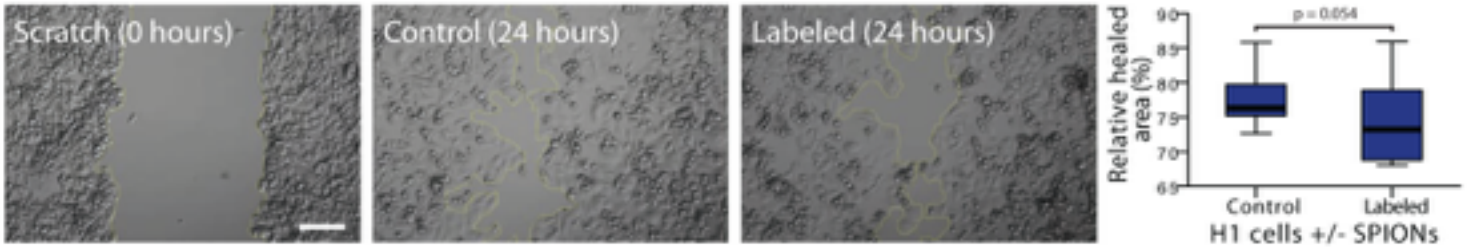


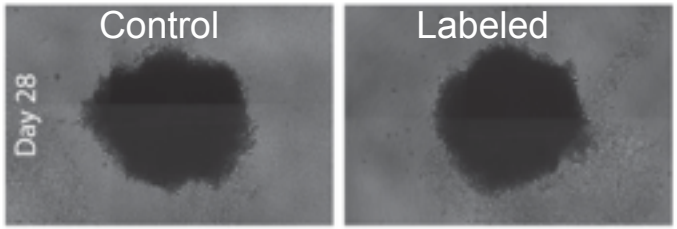
Prelabeling brain metastasis cells with low dose SPIOs

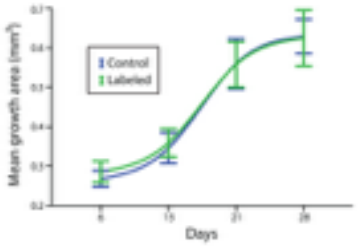




10 ug/mL

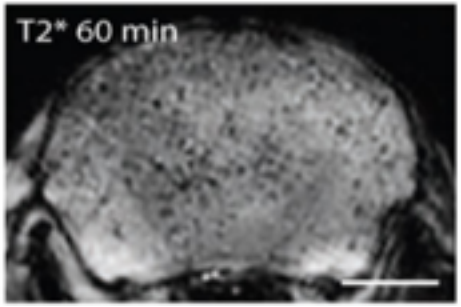


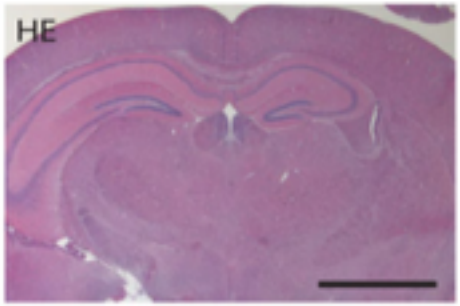


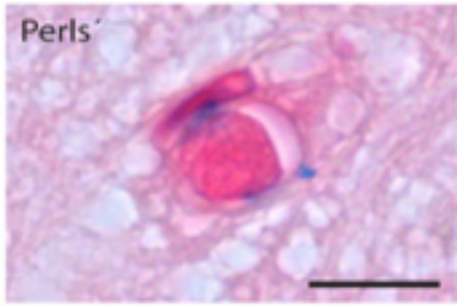


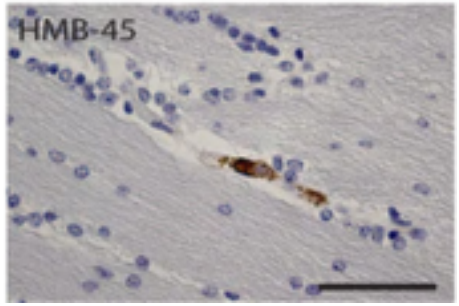
Sundstrøm T, …, Thorsen F, Cancer Res 2013

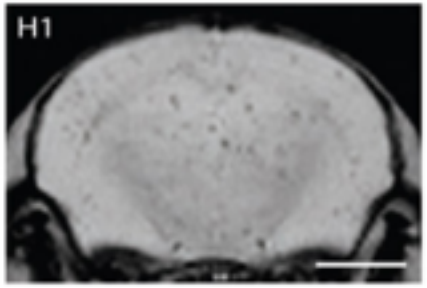
Single prelabeled cells can be detected *in vivo* by MRI

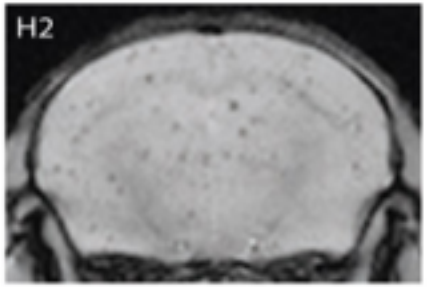


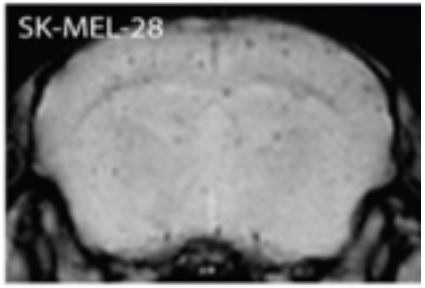


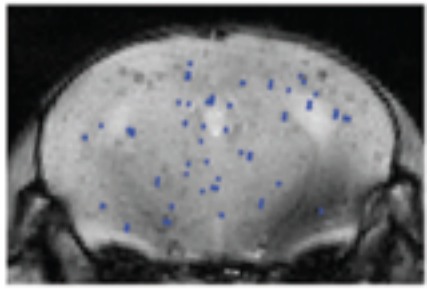


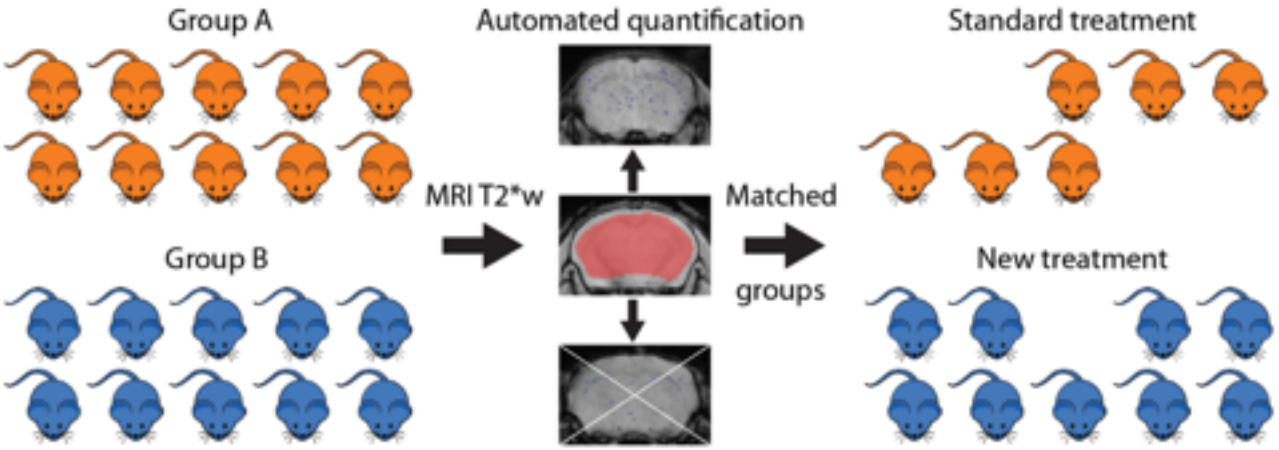


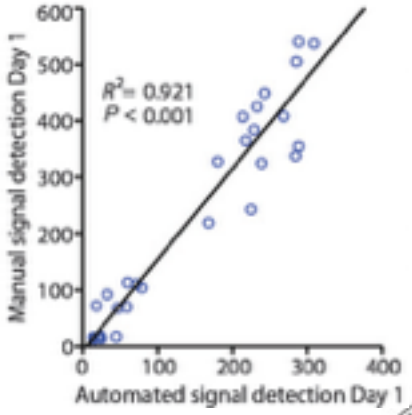








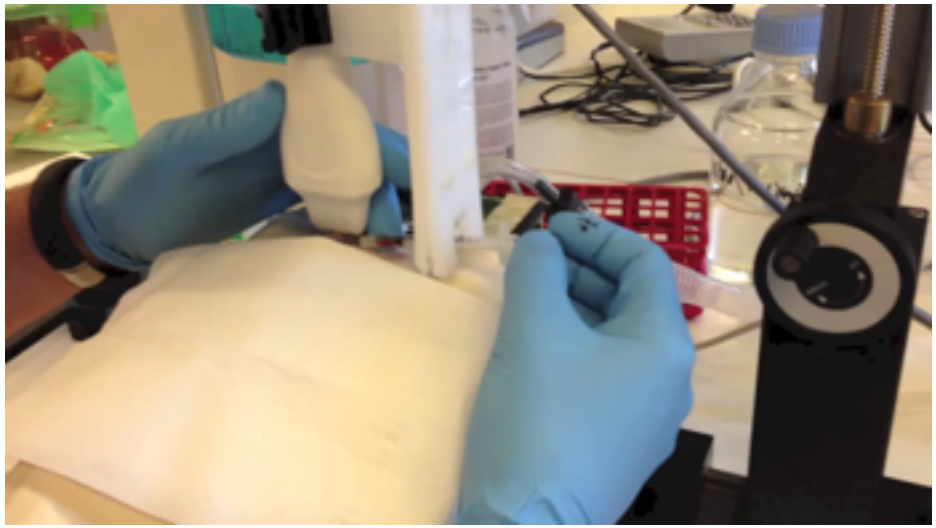


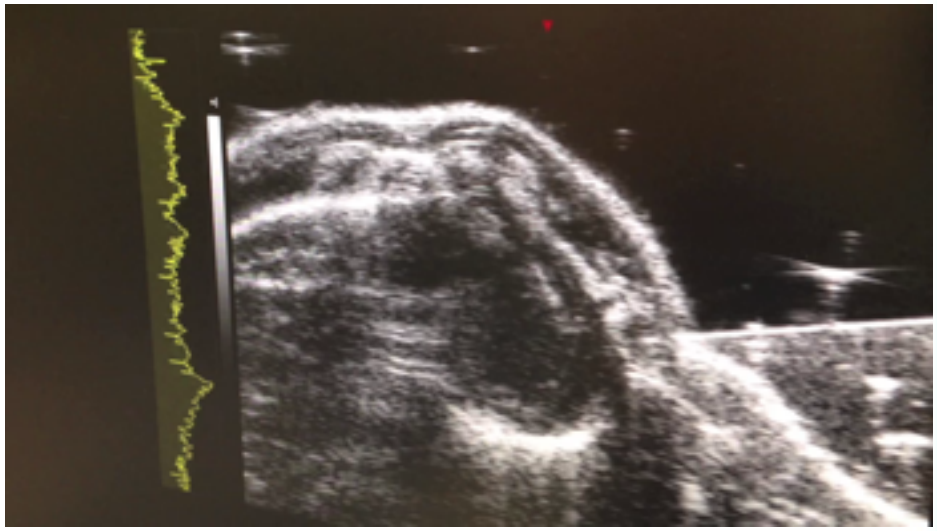


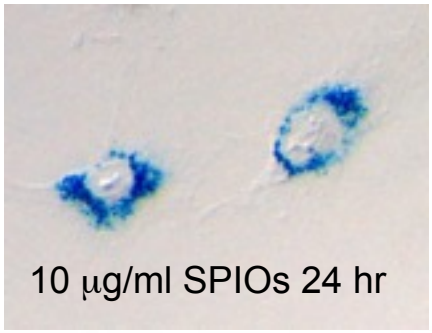
Sundstrøm T, …, Thorsen F, Cancer Res 2013

Ultrasound guided injections of prelabeled cells

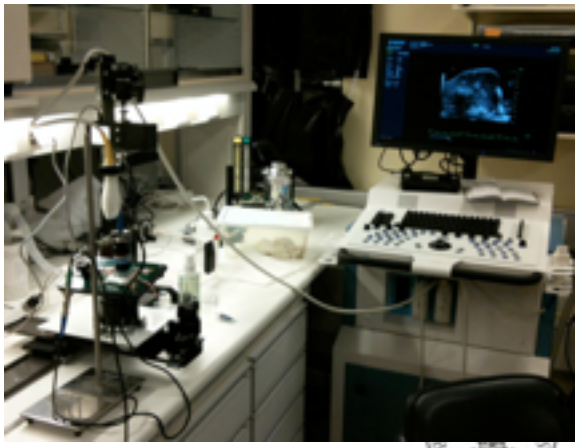
5 X 10^5 H1_DL2 melanoma brain metastases cells are injected into the left cardiac ventricle.







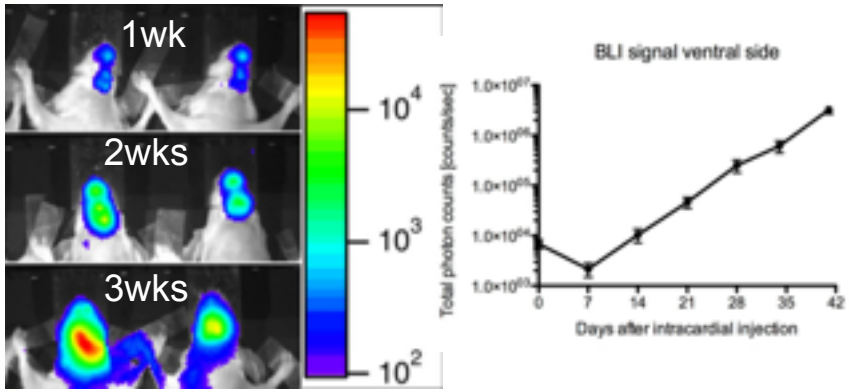
Vevo 2100 small animal ultrasound scanner. (Visualsonics, Toronto, Ontario, Canada).



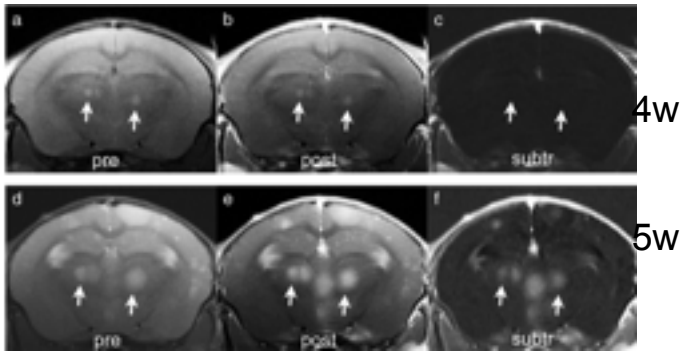
Sundstrøm T, …, Thorsen F, Neuro-Oncol 2014

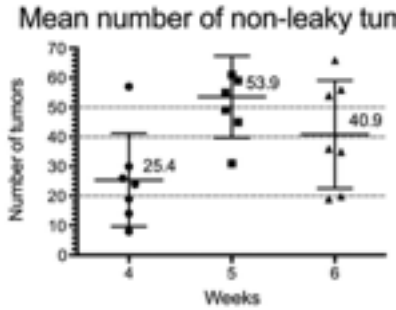
Study of BBB using different sized contrast agents

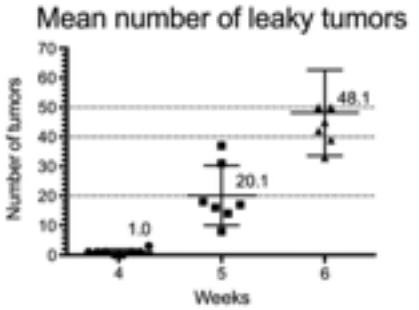
D-Luciferin (MW 302Da): 1 week



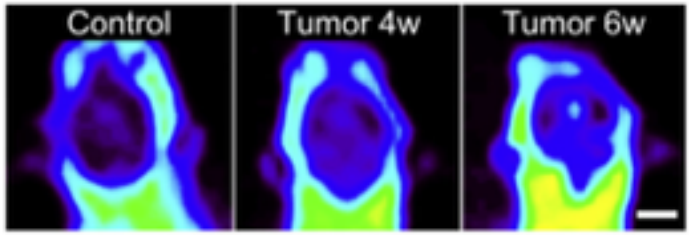
Prohance (566Da), C3 (2066Da)

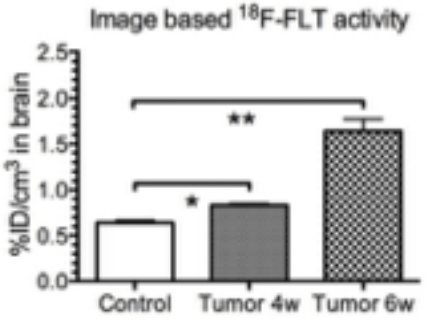




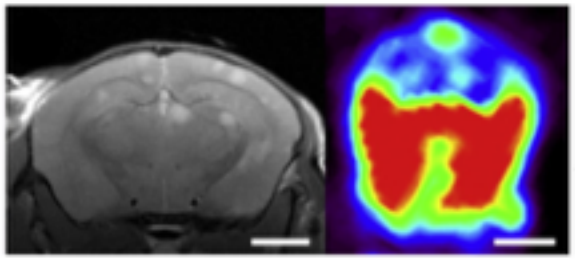


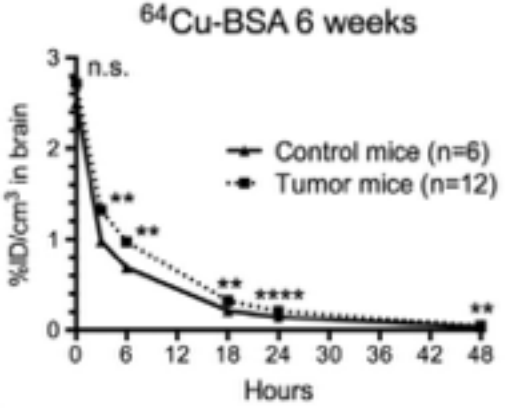
^{18}F-FLT (MW 244Da): 4 weeks





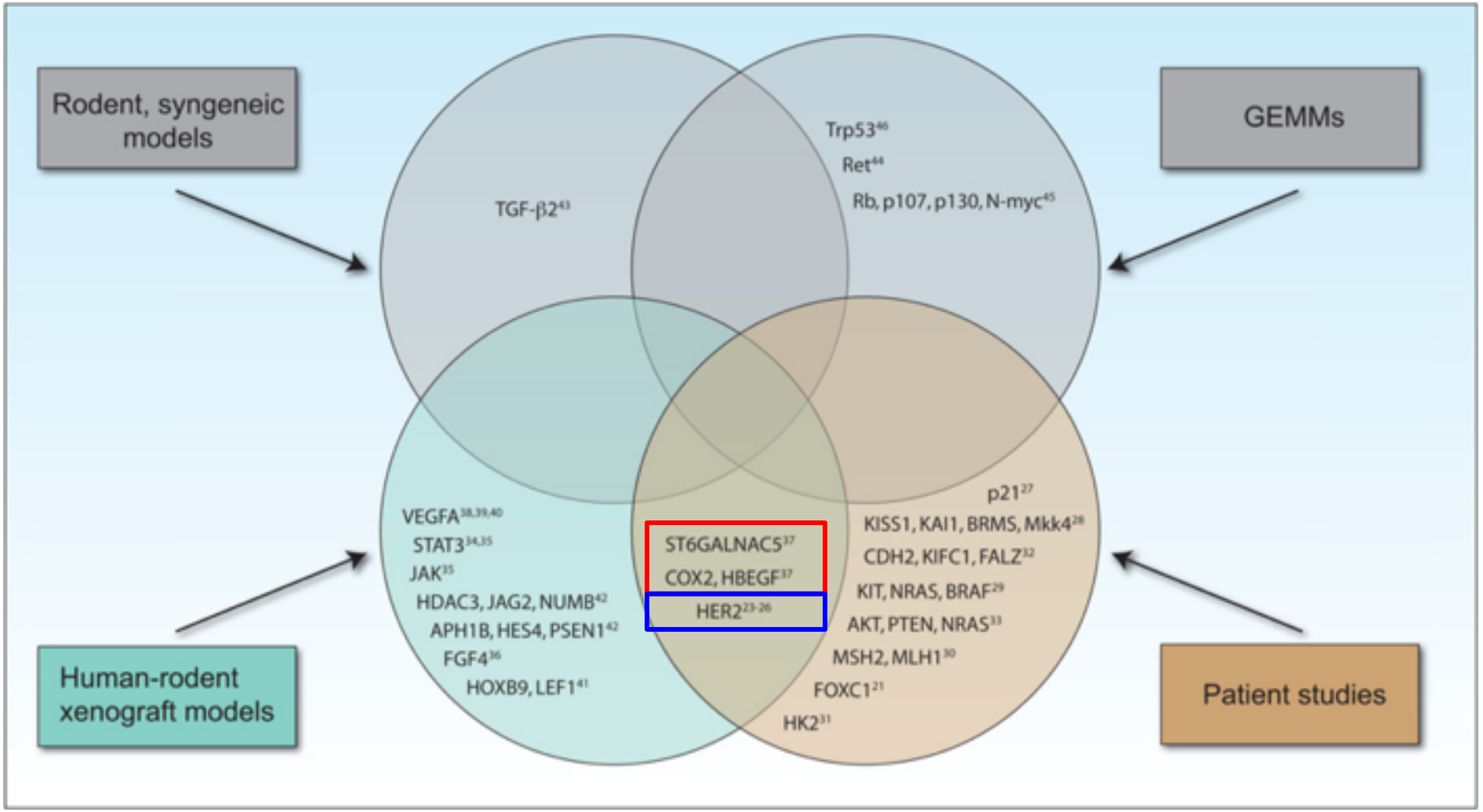
^{64}Cu-BSA (65.55kDa): 6 weeks





Thorsen F et al, J Contr Rel 2013

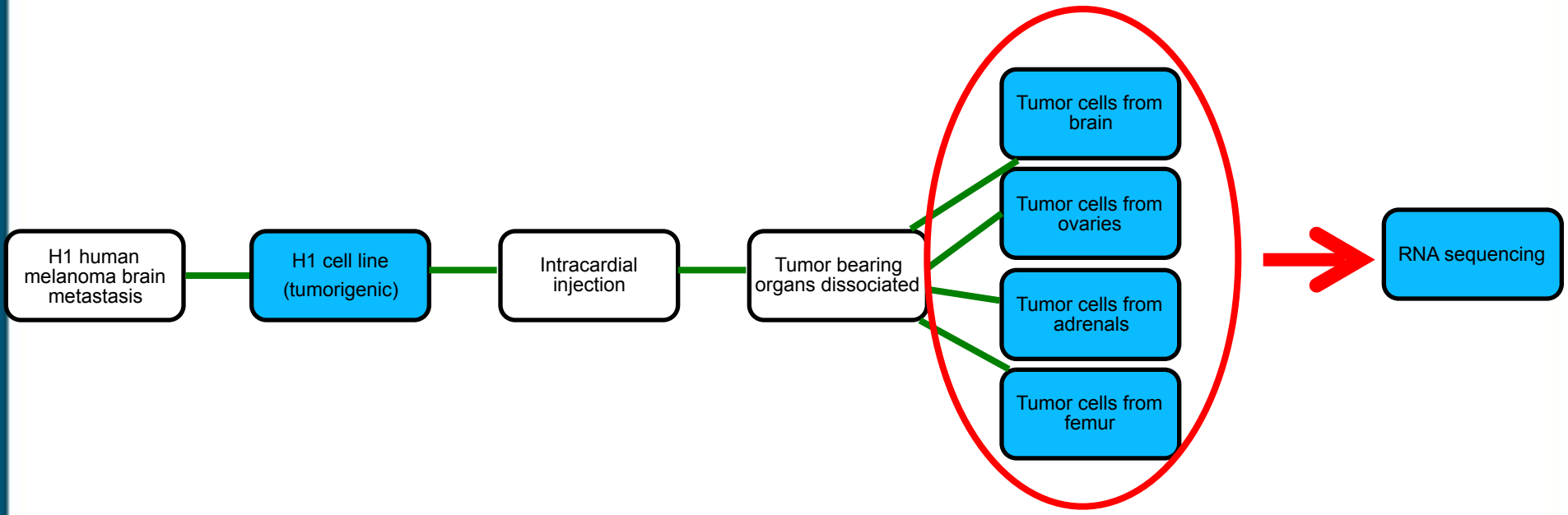
Genes involved in brain metastasis

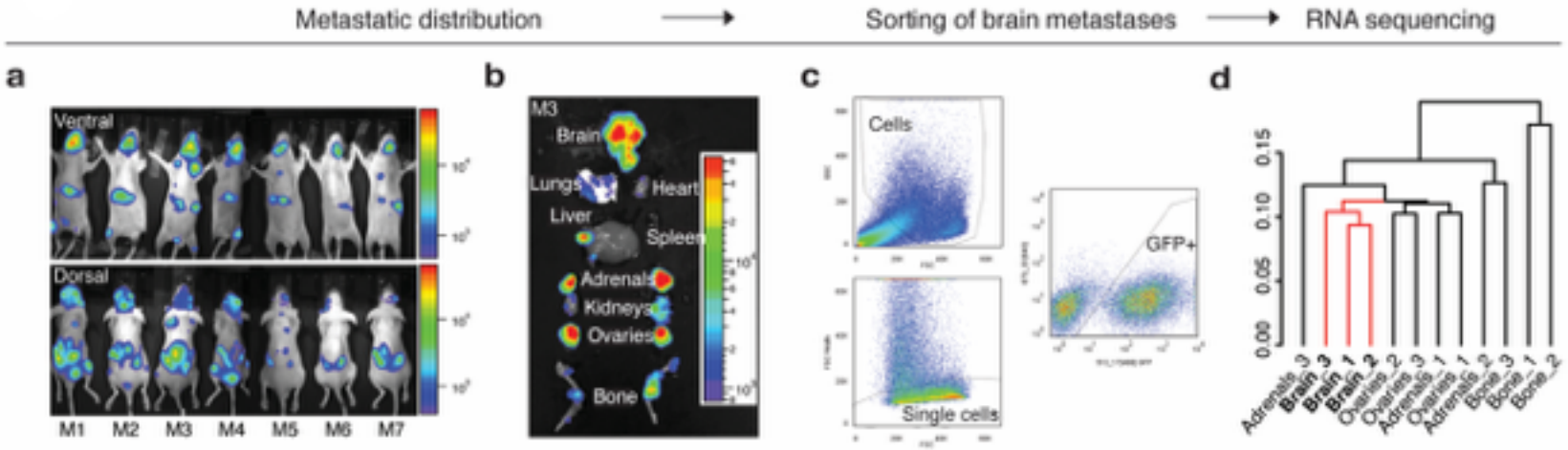


Bos PD et al, Nature 2009
Stemmler HJ et al, Breast 2006

Daphu I, …, Thorsen F. Clin Exp Metastasis 2013

Is there a specific gene signature for melanoma brain metastases, compared to tumors in other organs?





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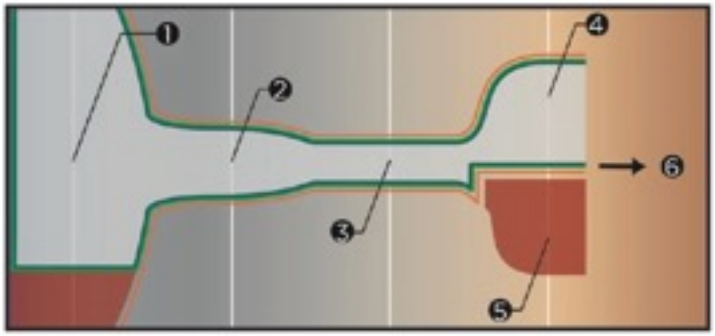
Determination of our 108-gene signature



(1) 134 differentially expressed genes between brain and any of the other organs - 122 were upregulated in brain and 54 were downregulated (some appeared in multiple lists)

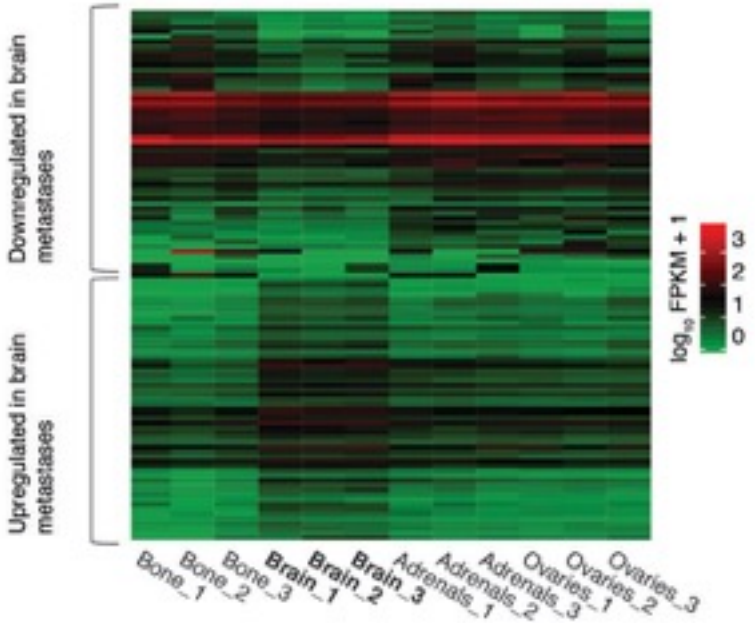
(2) 10 genes specific to brain metastases was selected using prediction analysis for microarrays (PAM) – all 10 genes were upregulated

(3) 8 of these 10 upregulated genes were validated using (A) a supervised rank product analysis of brain metastases vs. metastases to all the other organs pooled together, (B) a meta-analysis of rank product analyses of brain metastases vs. each individual organ, and (C) a significance analysis of microarrays (SAM)

(4) 46 genes with similar expression profiles to these 8 genes were selected using hierarchical clustering – these 54 upregulated genes were used for further analysis

(5) We selected the 54 top downregulated genes from a supervised rank product analysis of brain metastases vs. metastases to all the other organs pooled together

(6) A list of 108 genes (54 upregulated and 54 downregulated) was used for analysis with Connectivity Map



Sundstrøm T, …, Thorsen F, Manuscript

SCIENCE VOL 313 29 SEPTEMBER 2006

The Connectivity Map: Using Gene-Expression Signatures to Connect Small Molecules, Genes, and Disease

Justin Lamb,[1*] Emily D. Crawford,[1†] David Peck,[1] Joshua W. Modell,[1] Irene C. Blat,[1] Matthew J. Wrobel,[1] Jim Lerner,[1] Jean-Philippe Brunet,[1] Aravind Subramanian,[1] Kenneth N. Ross,[1] Michael Reich,[1] Haley Hieronymus,[1,2] Guo Wei,[1,2] Scott A. Armstrong,[2,3] Stephen J. Haggarty,[1,4] Paul A. Clemons,[1] Ru Wei,[1] Steven A. Carr,[1] Eric S. Lander,[1,5,6] Todd R. Golub[1,2,3,5,7*]

BROAD INSTITUTE

CONNECTIVITY MAP 02

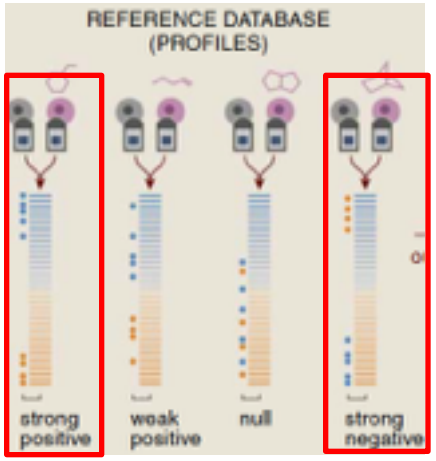
http://www.broadinstitute.org/cmap/

Contains more than 7000 expression profiles involving 1309 compunds.
Treatment vs. control experiments followed by microarray analysis on MCF7 (breast), ssMCF7 (breast), HL60 (leukemia), PC3 (prostate) and SKMEL5 (melanoma) cell lines.

Input

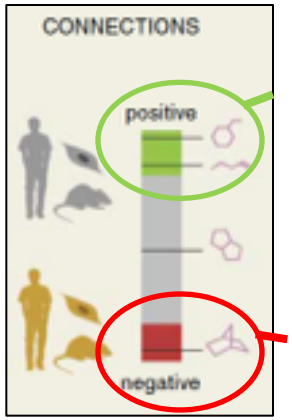
Signature investigated (brain metastasis gene signature)

cMAP



Ranked signature -drug matches

Output



Induce signature

Induce opposite signature

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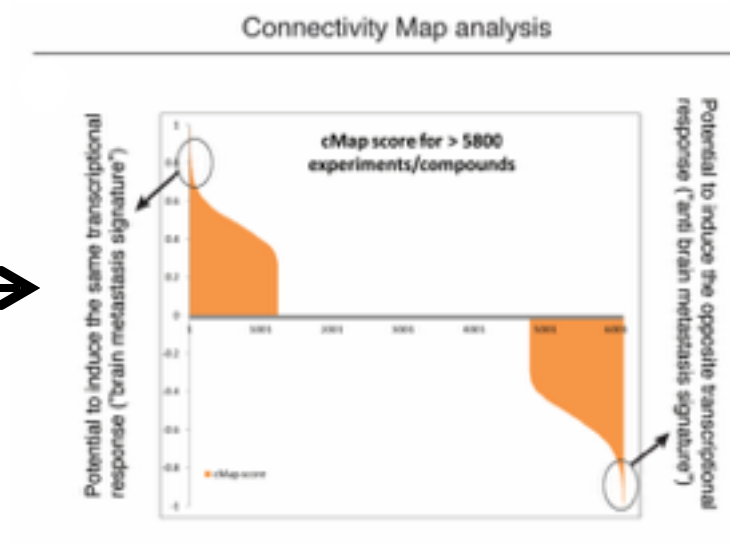
Genes involved, and top ten drug candidates

CBS	KIF5C	S100A4	TFF3
IFI44L	PARP14	HLA-DRB1	MIR210HG
MX1	MYADM	RNU2-2	S100A3
PRSS56	PTP4A3	RN7SL2	CAPS
XAF1	PLSCR1	ANGPTL4	CITED1
ASNS	ZNF140	SPAG4	RP11-151D14.1
MDK	IRF9	RN7SK	CCL3
CMPK2	SOGA3	J01415.23	LRRC32
PARP12	AP001610.5	TFF2	AC009469.1
TFRC	OAS3	S100A6	TGFBI
SCG3	IFIT3	RP11-1143G9.4	ADAM19
DDX58	CDC42EP1	RNY3P8	MT1DP
DCUN1D4	ASS1	RPPH1	RPL9P8
STAT1	HERC6	GSTA1	ITM2A
PARD3B	ACTG2	AP003419.11	AP000580.1
EPSTI1	MX2	SOX2	S100A13
RSAD2	RP11-820L6.1	HAPLN1	VOPP1
DDX60	OAS2	FAM69C	RP11-34H11.3
IFI44	IFIT1	MGP	TIMP3
HERC5	PPP1R16B	RNU11	HIST1H4C
KIAA1644	SARDH	MRPS6	RP11-872D17.8
ARSG	ARHGEF4	FLNB	CTD-2369P2.12
CAMK2D	C16orf89	Z98256.1	ECM1
SPARCL1	GATM	S100A1	SNORD3A
DTX3L	RP5-1061H20.4	ADM	CYR61
HEY1	SHISA2	MFI2	CNFN
SLFN5	ZNF853	RPL9P9	HTRA1



Drug candidates

Compound	CAS	Dose (μM)	cMap score	MW (g/mol)
Meticrane	1084-65-7	15	-1	275.34
Tolazamide	1156-19-0	13	-0.989	311.4
β-sitosterol	83-46-5	10	-0.984	414.7
Memantine	41100-52-1	19	-0.971	215.77
Niridazole	61-57-4	19	-0.967	214.22
Valproic acid	1069-66-5	200	-0.966	166.2
Letrozole	112809-51-5	14	-0.965	285.31
Todralazine	3778-76-5	15	-0.96	268.7
Thiostrepton	1393-48-2	2	-0.932	1664.8
Levocabastine	79547-78-7	9	-0.927	456.98

Cholesterol reducing

Alzheimer disease

Aromatase inhibitor

Antibiotic

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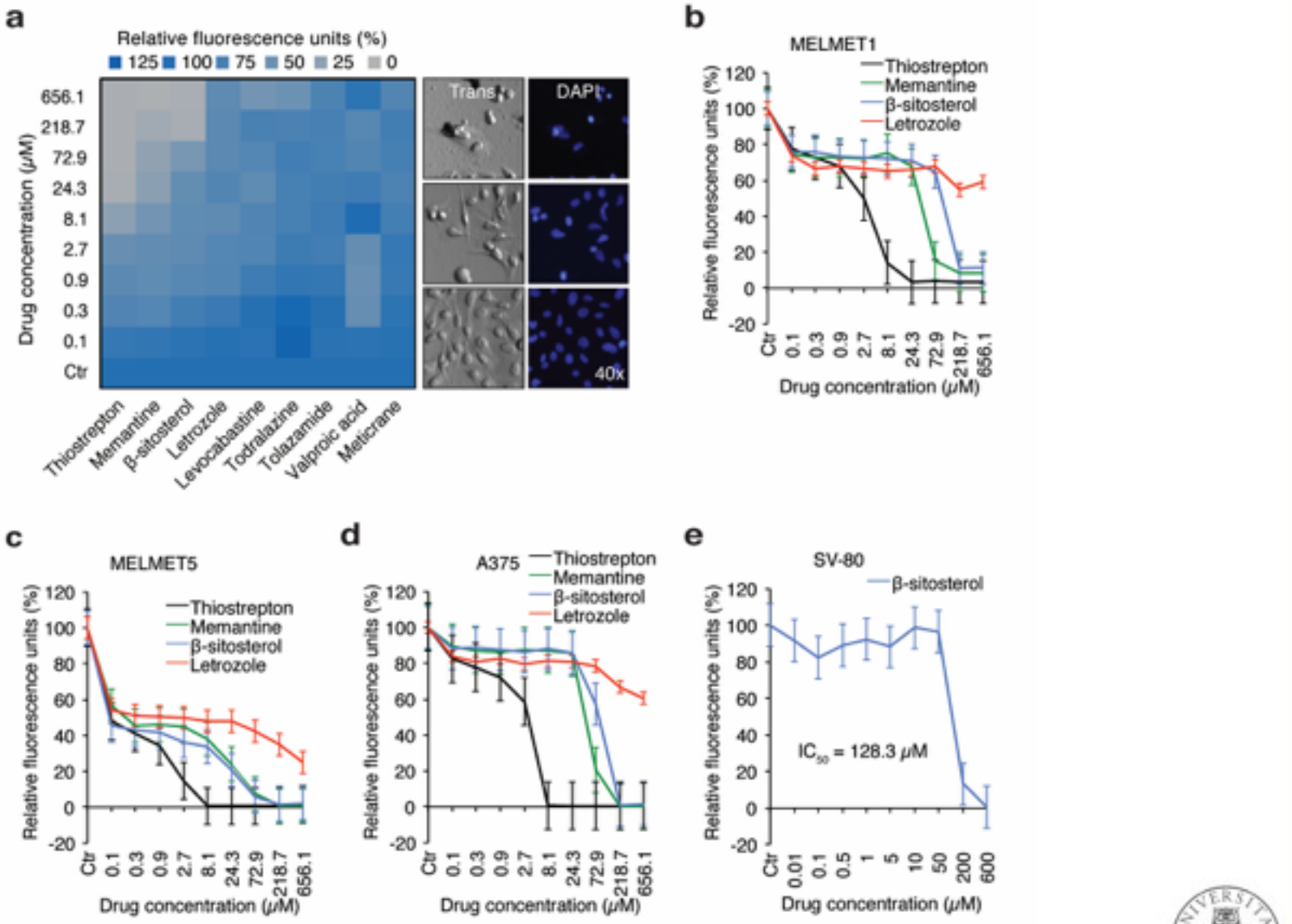
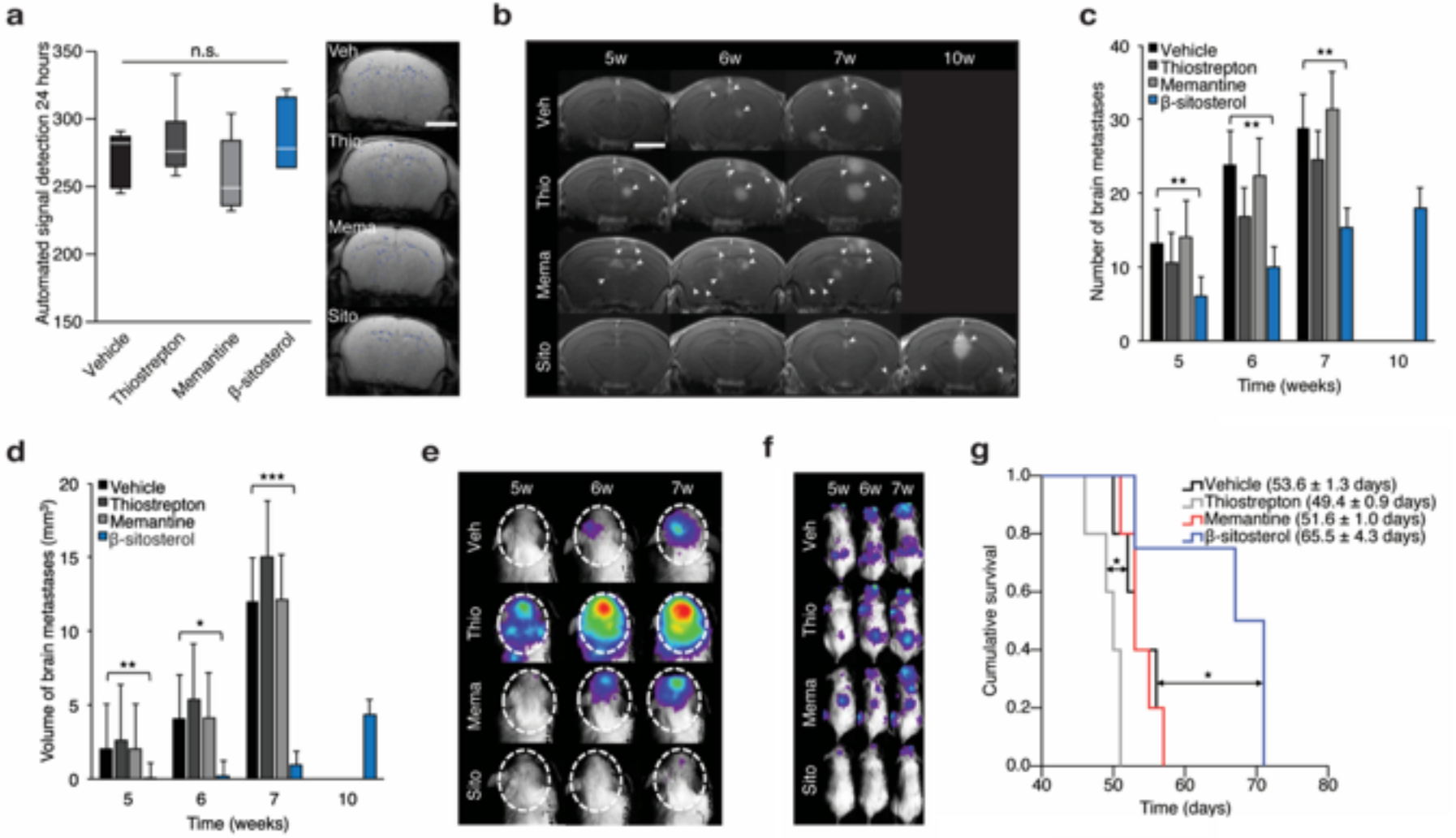
In vitro screening of drug candidates



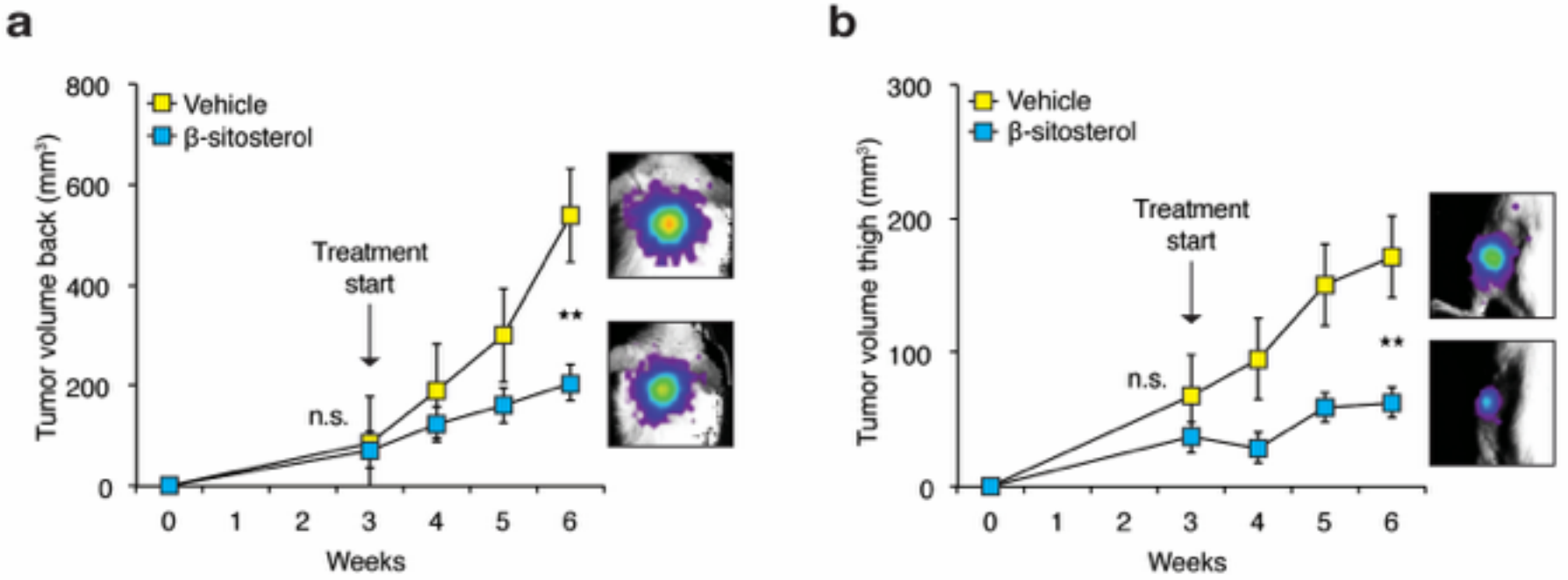
Sundstrøm T, …, Thorsen F, Manuscript

Screening 3 drugs *in vivo* using H1_DL2 cells



Sundstrøm T, …, Thorsen F, Manuscript

Treatment of subcutaneous melanoma tumors



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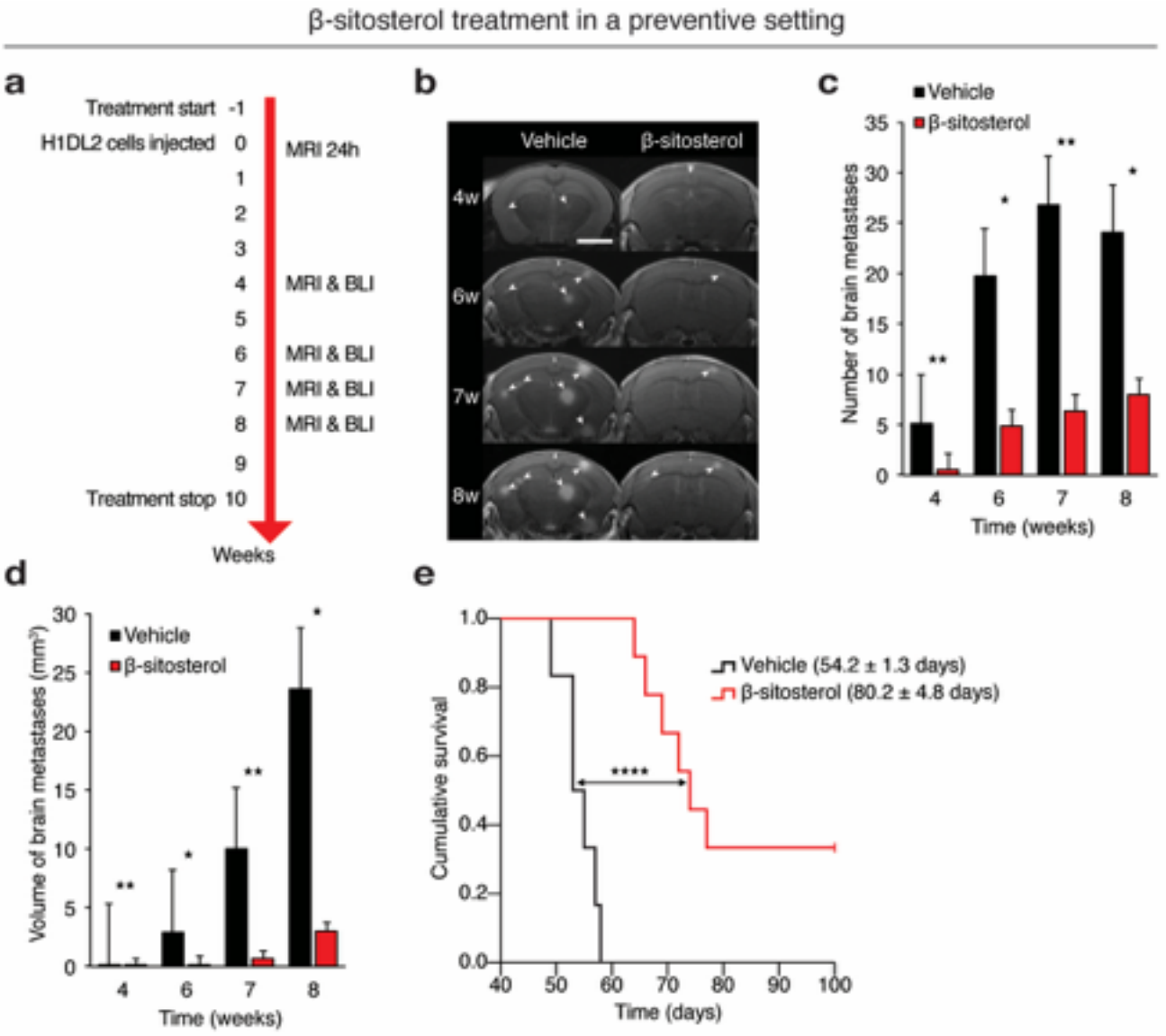
Treating with β-sitosterol in a preventive setting



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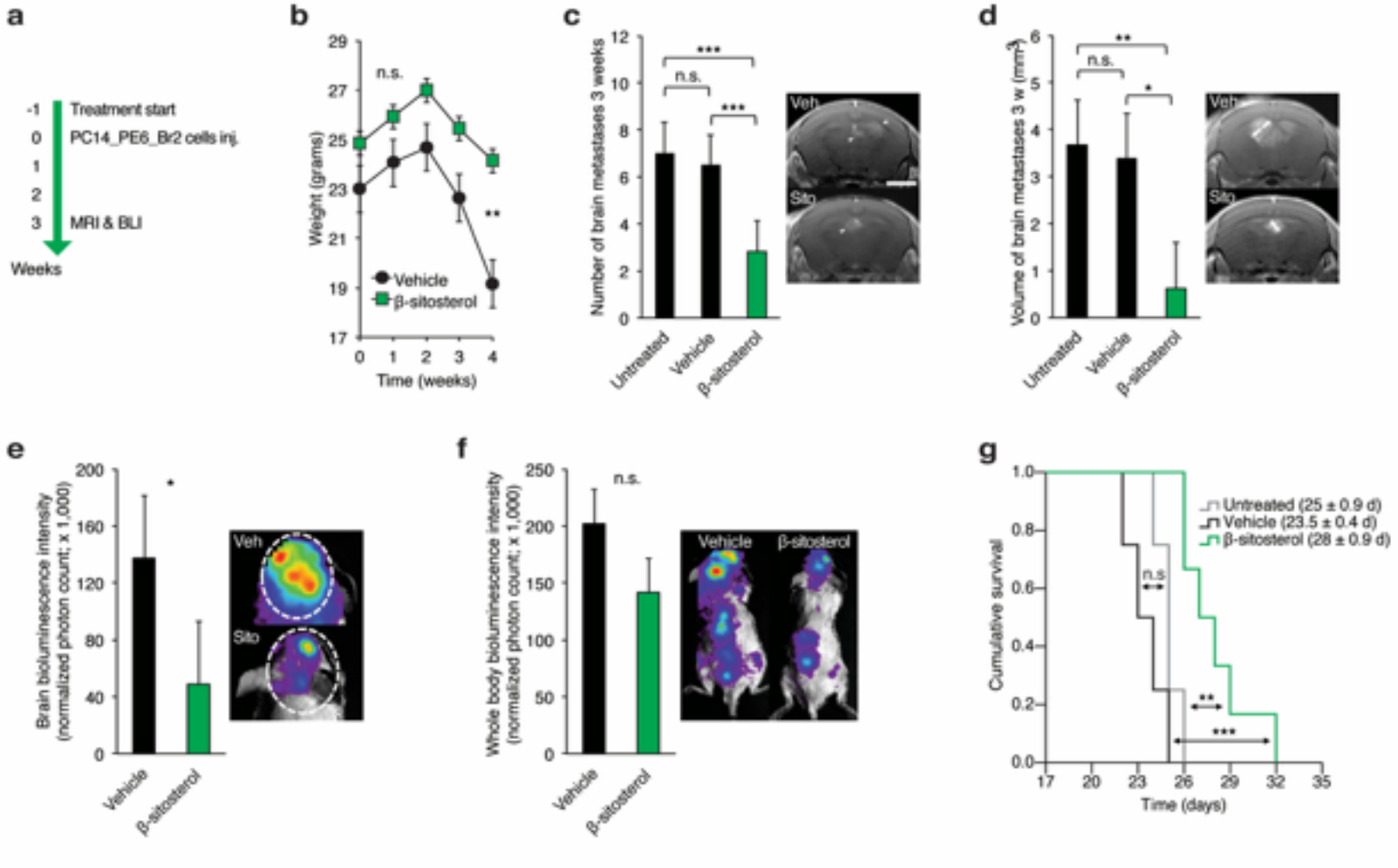
Treatment of lung adenocarcinoma brain mets



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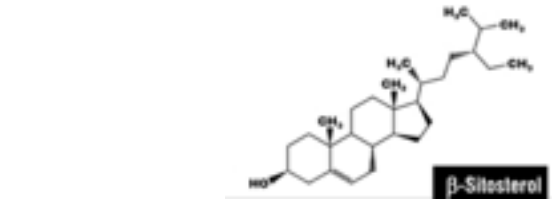
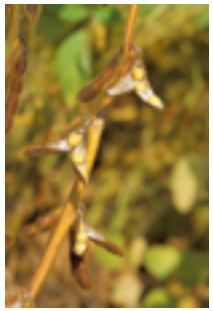
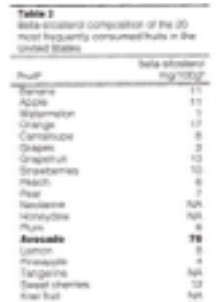
β-sitosterol ($C_{29}H_{50}O$)

- ✓ β-sitosterol is the most abundant phytosterol (plant sterol). The proportion of sterols in plants are 65% β-sitosterol, 30% campesterol and 5% stigmasterol.
- ✓ MW 414.71 g/mol (crosses an intact BBB and accumulate) (Vanmierlo, 2012).
- ✓ Structurally similar to cholesterol (extra ethyl group).
- ✓ Cannot be synthesized by humans.
- ✓ Found in many plants, seeds and fruits, e.g. wheat germ, corn oils, soybeans, rice bran, saw palmetto, avocados, tree nuts.
- ✓ Used as an additive in cosmetic products, and as a nutritional supplement.







Duester JADA 2001

β-sitosterol – consumption and absorption

- ✓ **Phytosterol consumption** (Messina JNCI 1991; Ostlund ARN 2002)
  - ✓ Asians and vegetarians 345-400 mg/day
  - ✓ Western societies 80 mg/day

  - ✓ **Uptake and transport**
  - ✓ Takes the place of cholesterol in the intestinal micelles (Moreou PLR 2002)
  - ✓ Competes with cholesterol for uptake via the intestinal NPC1L1 transporter (Davis JBC 2004)
  - ✓ Most β-sitosterol is re-secreted in the intestine (Graf JCI 2002; Yu PNAS 2002)
  - ✓ Some β-sitosterol is transported by lipoproteins and incorporated into cell membranes (Awad JN 2000)

- ✓ **Absorption**
  - ✓ β-sitosterol <5% and cholesterol 45-54% (Salen JCI 1970)
  - ✓ β-sitosterol plasma levels < 1 mg/dL (Graf JCI 2002; Yu PNAS 2002; Yu JCI 2002)
  - ✓ 2-3 g β-sitosterol → 30% increase in serum levels (Hallikainen EJCN 2000; Weststrate EJCN 1998)

- ✓ **Bioavailability in healthy subjects** (Duchateu DMD 2012)
  - ✓ Oral administration
  - ✓ Metabolic turnover 5.8 mg/d (6-12 mg/d in Salen JCI 1970)
  - ✓ Half-life 72.4h (mean T1/2)
  - ✓ Regarded as “safe” by FDA (3 g/day for 3 years).

Nut consumption and health effects

- ✓ **Reduction in mediators of chronic disease**
  - ✓ Oxidative stress (Jenkins JN 2006; Torabian JHND 2009)
  - ✓ Inflammation (Jiang AJE 2006)
  - ✓ Blood cholesterol (Sabaté AIM 2010)
  - ✓ Visceral adiposity (O'Neil JACN 2011)
  - ✓ Hyperglycemia (Jenkins JN 2006; Jiang AJE 2006; Jenkins DC 2011)
  - ✓ Insulin resistance (Casas-Agustench NMCD 2011; Tapsell EJCN 2009)
  - ✓ Endothelial dysfunction (Ma DC 2010)

- ✓ **Increased nut intake associated with reduced risk of**
  - ✓ Coronary heart disease (Kris-Etherton JN 2008)
  - ✓ Major cardiovascular events (Estruch NEJM 2013)
  - ✓ Diabetes mellitus type 2 (Jiang JAMA 2002; Villegas AJCN 2008; Pan JN 2013)
  - ✓ Metabolic syndrome (Fernández-Montero PHN 2013)
  - ✓ Weight gain/obesity (Mozaffarian NEJM 2011; Salas-Salvadó AIM 2008; Bes-Rastrollo Obesity 2007)
  - ✓ Colon cancer (Singh AJE 1998)
  - ✓ Hypertension (Tsai AJE 2004)
  - ✓ Gallstone disease (Tsai AJE 2004; Idem AJCN 2004)
  - ✓ Diverticulitis (Strate JAMA 2008)
  - ✓ Death from inflammatory diseases (Gopinath AJCN 2011)

- ✓ **Nut consumption and reduced mortality from heart/cardiovascular disease, cancer** (Goldstein AIM 1992; Fraser 1997; Mann Heart 1997; Ellsworth NMCD 2011; Baer AJE 2011; van den Brandt AJCN 2011)

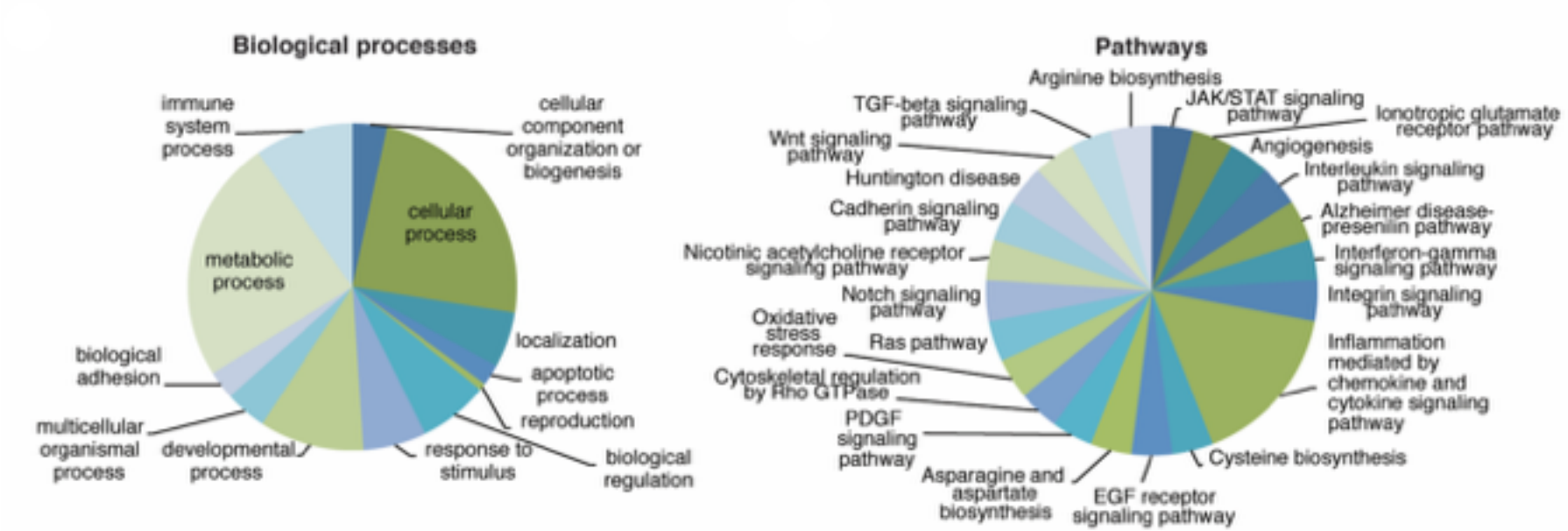
β-sitosterol – epidemiological studies

- ✓ Phytosterols reduce breast cancer risk (Torres-Sanchez PHN 2009; Cui CEBP 2007)
- ✓ High plant sterol intake reduces lung cancer risk (Mendilaharsu LC 1998)
- ✓ High phytosterol intake associated with reduced stomach cancer risk (De Stefani NC 2000)
- ✓ Plant sterols not associated with lower risk of colon and rectal cancers (Normén AJCN 2001)

β-sitosterol – experimental *in vivo* data

- ✓ MCF-7 breast cancer cells: Reduced xenograft growth (Ju JN 2004)
- ✓ MDA-MB-231 breast cancer cells: Reduced xenograft growth (Awad AR 2000; Ju JN 2004)
- ✓ 4T1 breast cancer cells: Reduced xenograft growth and increased survival (Kazlowska EBCAM 2013)
- ✓ NMBA-induced oesophagus cancer: Reduced tumor formation and progression (Stoner NC 2006)
- ✓ B16BL6 melanoma cells: Reduced metastasis (Imanaka BPB 2008)
- ✓ Unknown breast cancer cells: Reduced metastasis (Qin PC 2011)
- ✓ PC-3 prostate cancer cell: Reduced metastasis (Awad EJCP 2001)
- ✓ Apc^{Min} colon mice: Induced adenoma formation (Marttinen JNB 2013; Marttinen NC 2014)

Biological processes and signaling pathways



Protein Analysis Through Evolutionary Relationships (PANTHER) classification system.

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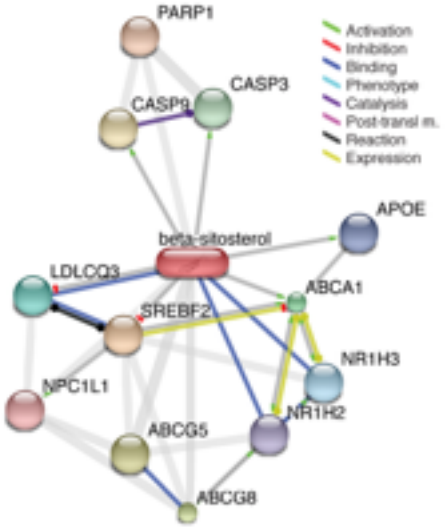
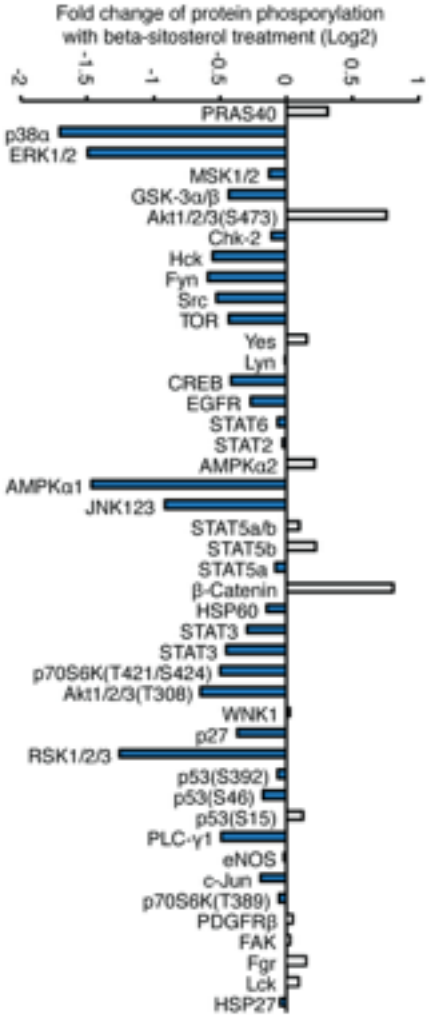
Protein interactions and biological processes– β-sitosterol





Biological process	*p*-value
Cholesterol homeostasis	0
Protein amino acid ADPribosylation	0
Heterocycle metabolism	0
Nuclear transport	0
Lipid transport	0
DNA recombination	4.095×10^{-5}
Interphase	0.0006142
Regulation of DNA metabolism	0.0006552
Chromosome segregation	0.001843
Mitochondrial transport	0.002634
Transcription initiation	0.005078
Chromosome condensation	0.006907
Meiotic cell cycle	0.02992

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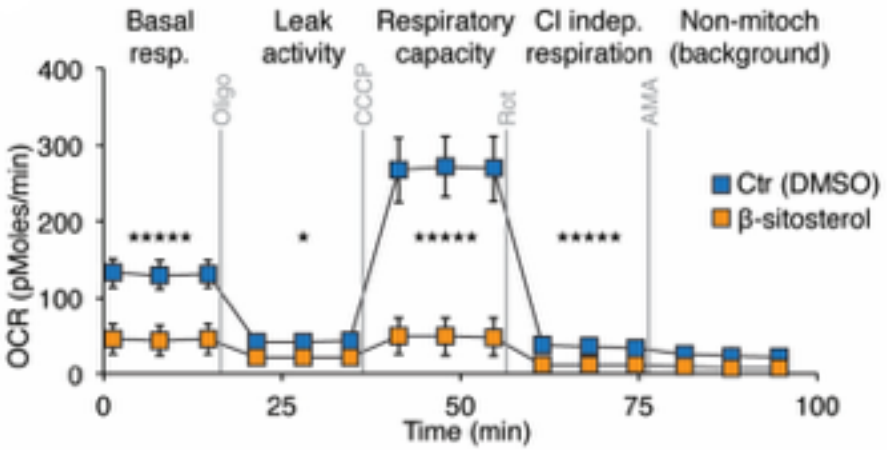
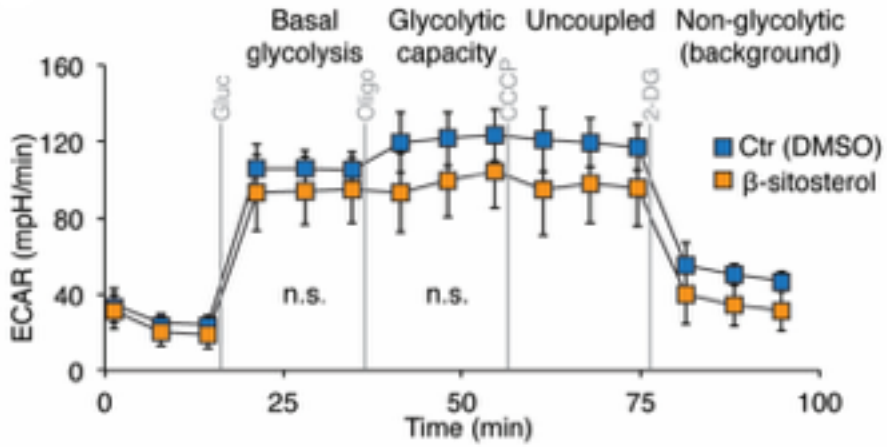
β-sitosterol targets mitochondrial respiration through Complex I inhibition

Basal respiration



Suggests that Complex I is a likely target for β-sitosterol

Basal glycolysis



Basal glycolysis and glycolytic capacity unaffected by β-sitosterol

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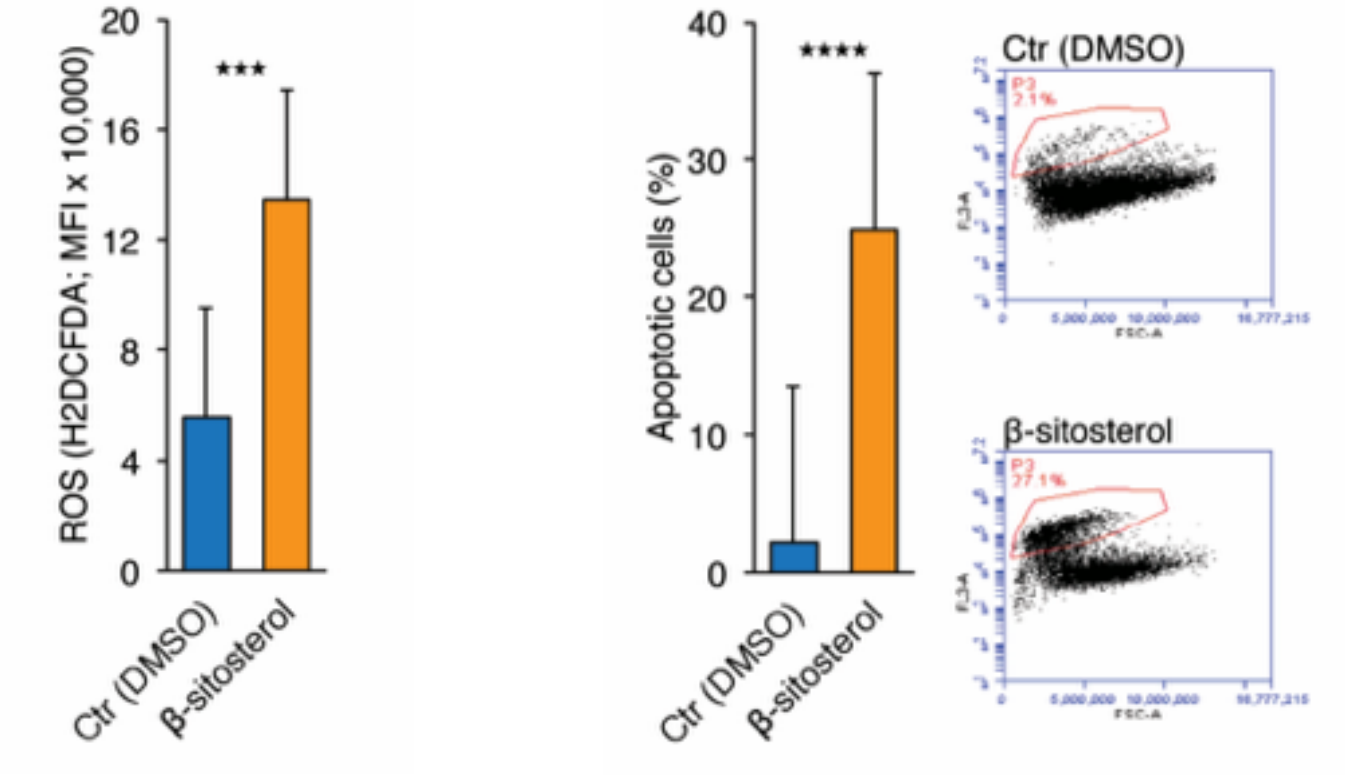
β-sitosterol stimulates ROS production and induces apoptosis



Mitochondria are major producers of ROS.

Inhibition of CI is known to stimulate ROS production

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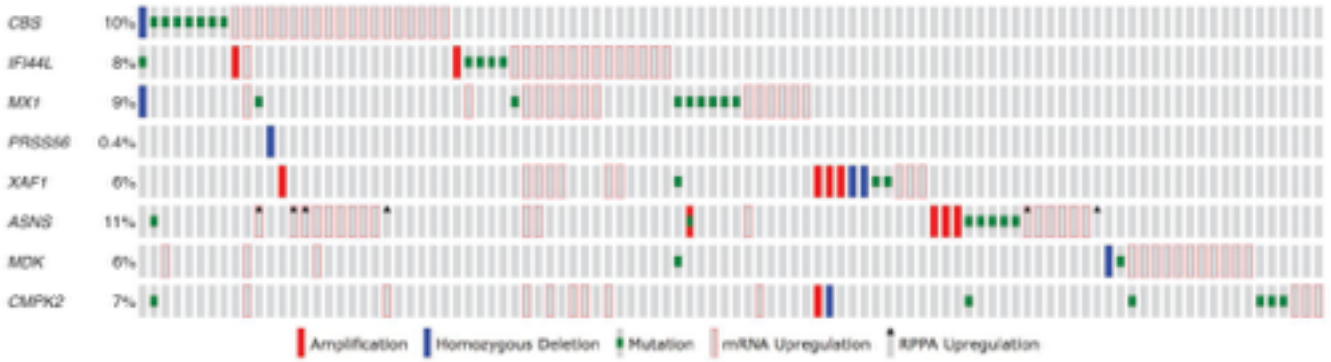
Clinical relevance of brain metastasis signature



cbioportal.org

TCGA dataset
278 mel. pat.

Top 8 genes-11%

Prognostic model	Dataset	Expression input	*P*-value	HR [CI]
(1) Potential prognostic utility of signature genes				
1	TCGA	*CBS*	0.03	1.13 [1.01-1.27]
2	TCGA	*PARD3B*	0.04	0.87 [0.76-0.99]
3	TCGA	*HEY1*	0.02	1.23 [1.04-1.45]
4	TCGA	*CDC42EP1*	0.02	1.2 [1.03-1.4]
5	TCGA	*MX2*	0.04	0.88 [0.79-0.99]
6	TCGA	*ITM2A*	0.03	0.86 [0.76-0.99]
7	TCGA	Sum of genes	0.63	0.89 [0.57-1.41]
(2) Potential prognostic utility of putative β-sitosterol targets				
1	TCGA	*CASP3*	0.01	0.66 [0.47-0.92]
2	GSE19234	*NR1H3*	0.05	0.53 [0.29-0.99]
3	GSE19234	*ABCG5*	0.007	2.75 [1.32-5.71]
4	GSE19234	*PARP1*	0.01	5.4 [1.45-20.13]
5	TCGA	Sum of genes	0.78	0.95 [0.68-1.34]
(3) Potential prognostic utility of genes coding (array) proteins responding to β-sitosterol				
1	TCGA	*AKT2*	0.04	1.6 [1.02-2.5]
2	TCGA	*LCK*	0.01	0.89 [0.81-0.97]
3	TCGA	*LYN*	0.01	0.78 [0.65-0.94]
4	TCGA	*MAPK9*	0.007	0.59 [0.41-0.87]
5	TCGA	*RORC*	0.01	0.94 [0.9-0.99]
6	TCGA	*RPS6KA3*	0.004	0.69 [0.53-0.89]
7	GSE19234	*AKT1*	0	14.07 [4.1-48.28]
8	GSE19234	*CDKN1B*	0.006	0.26 [0.1-0.68]
9	GSE19234	*HSPB1*	0.05	2.84 [1-8.04]
10	GSE19234	*HSPD1*	0.02	2.46 [1.15-5.29]
11	GSE19234	*LCK*	0.03	0.66 [0.45-0.95]
12	GSE19234	*MAPK1*	0.006	0.26 [0.1-0.68]
13	GSE19234	*MAPK14*	0.003	5.39 [1.76-16.52]
14	GSE19234	*STAT3*	0.01	0.42 [0.21-0.82]
15	GSE19234	*STAT5A*	0.04	0.45 [0.21-0.95]
16	GSE19234	*YES1*	0.04	1.7 [1.02-2.84]
17	TCGA	Sum of genes	0.13	0.58 [0.29-1.17]
18	TCGA	Sum of genes coding down-regulated proteins	0.12	0.55 [0.26-1.18]
19	TCGA	Sum of genes coding up-regulated proteins	0.20	0.61 [0.28-1.31]

Prognostic potential:
PROGene system.

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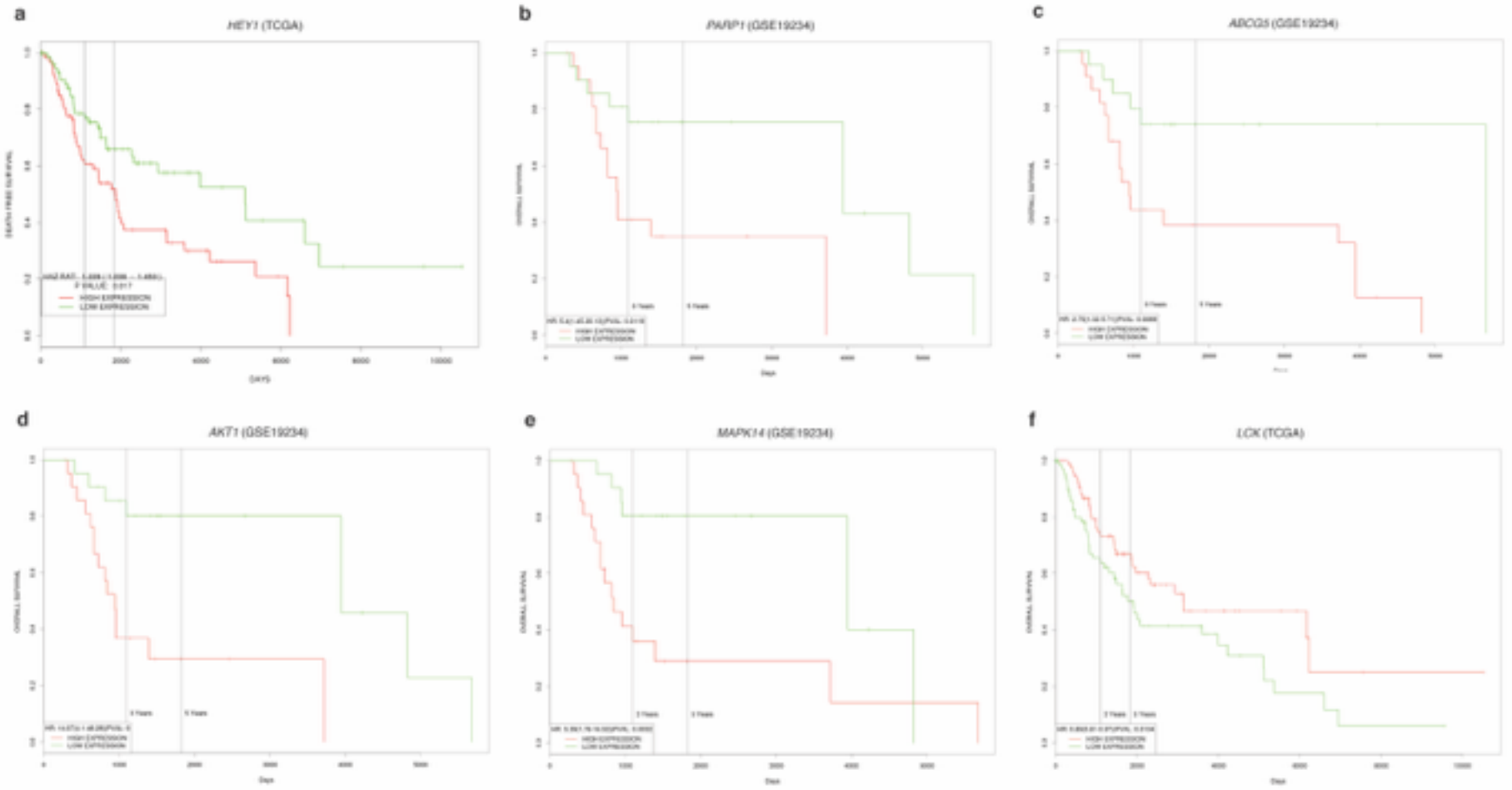
Associations between gene expression and survival



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Conclusions

We have developed representative animal models for studying brain mets.

In our models, the BBB breaks down late in tumor development.

A 108-gene brain metastatic gene signature was found.

Based on this signature, several drugs currently used for treating other conditions than cancer were predicted to have anti-metastatic effects.

In particular, β-sitosterol were shown inhibit brain metastatic growth.

β-sitosterol extensively suppressed the MAPK pathway.

β-sitosterol reduced mitochondrial respiration.

Our results strongly encourages further assessment of β-sitosterol as an adjuvant to established MAPK-targeted therapies.

Acknowledgments

Department of Biomedicine, University of Bergen
Rolf Bjerkvig
Kai-Ove Skaftnesmo
Karl Johan Tronstad
Gro Vatne Røsland
Lars Prestegarden
Jobin Varughese

Department of Neurosurgery, Haukeland University Hospital
Terje Sundstrøm
Morten Lund-Johansen

CRP Santé, Luxembourg
Francisco Azuaje

University of California Davis, USA
Katherine Ferrara
Clifford Tepper
Elizabeth Ingham
Lisa Even
Sarah Tam

Bergen Center for Computational Science, University of Bergen
Kjell Petersen

Research funding

University of Bergen

The Kristian Gerhard Jebsen Foundation

The Western Norway Regional Health Authorities

The Norwegian Cancer Society

The Research Council of Norway