Cancer Stem Cells: Dedifferentiation, Reprogramming & Transdifferentiation:

BCVS, July 14, 2014, Las Vegas
Namibia, 2011
THE WAR ON CANCER

I will also ask for an appropriation of an extra $100 million to launch an intensive campaign to find a cure for cancer, and I will ask later for whatever additional funds can effectively be used. The time has come in America when the same kind of concentrated effort that split the atom and took man to the moon should be turned toward conquering this dreaded disease. Let us make a total national commitment to achieve this goal. America has long been the wealthiest nation in the world. Now it is time we became the healthiest nation in the world.—President Richard M. Nixon in his 1971 State of the Union address.
Lessons learned in the last 40 Years:

- Cancer is not a single disease
- Cancer incidences increase with age
- Cancer is the disease of the genes
- Cancer is a multistep process
Hallmarks of Cancer

(Mantovani Nature 2009; an integration to Douglas Hanahan and Robert A. Weinberg Cell, 2000)
Mouse Models for Human Tumors

- REASONABLE PHENOTYPIC AND GENOTYPIC FACSIMILE
- AUTHENTICATE KNOWN CANCER GENES
- IDENTIFY ADDITIONAL CANCER GENES
- RECONSTRUCT PATHOGENIC PATHWAY
- EXPLORE MOLECULAR PATHOGENESIS
- CARCINOGEN TESTING
- PRECLINICAL TRIALS
Current Mouse Models of Human Cancer

- Mutagenesis
- Xeno- or Allografts
- Transgenic Animals
- Conditional Knock-out
Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is the most malignant of the primary brain tumors and is almost always fatal.

Nearly all GBM patients die of their disease with a median survival of 12 months, even if they had full treatments including surgery, radiotherapy and chemotherapy.

Malignant gliomas are among the most vascular of human tumors, making them especially attractive targets for angiogenesis inhibitors.
In fact 74% of glioblastomas sequenced harbored aberrations in all three pathways, thus confirming that alterations in these three pathways are a core requirement of glioblastoma formation.
GBM Mutations

H-RAS (activation)
Downstream of Growth factor receptors

PTEN (inactivation)
Leads to AKT Activation

NF-1 Mutation

p53 Mutation
Model Design

**Cre-inducible lenti-viral vector infection**

- **a** H-RasV12
- **b** Cre
- **c** Merge + DAPI
- **d** H-RasV12
- **e** Cre
- **f** Merge + DAPI

**pTomo-Ras virus infection into HeLa cells: MOI 10**

pTomo RasV12 lentivirus injection into hippocampus in wild type mouse

Flag RasV12 (Alexa 647)
pTomo RasV12 lentivirus injection into hippocampus in GFAP-Cre mouse

GFP

RFP

Flag RasV12 (Alexa 647)

Merge (Blue: DAPI)
Lentiviral Injections into Three Different Sites

Cortex

Hippocampus

Subventricular zone

OB: olfactory bulb  CTX: cortex  HP: hippocampus  LV: lateral ventricle  SVZ: subventricular zone
H-Ras and AKT Induced Tumor

- enlarged head
- irregularly enlarged cerebrum
- normal cerebellum

H-E staining

GFP
H-Ras and AKT Induced Tumor

High cellular density  Pseudopalisading

Necrosis  Perivascular infiltration

GFP  +DAPI

Invasion of the tumor cells into the normal tissues

H-E staining  Confocal
GBMs Are Invasive
Histological examination of tumors induced by H-Ras and AKT in GFAP-Cre mice

1. High cellular density: positive
2. Microvascular proliferation: positive
3. Necrosis within a densely proliferative region: positive
4. Infiltrative character: positive
5. Nuclear pleomorphism: negative
6. High mitotic activity: negative
LV-H-RAS-sip53 (pToDi) induced tumors in GFAP Cre+ WT mice

Tumor pathology

- A. High cellular density and nuclear pleomorphism
- A. Hemorrhage and vascularity
- C. Perivascular infiltration
- D. Pseudopalisading
- E. Infiltrative characteristic
The tumor contained the cells expressing glial (GFAP), Neuronal (Tuj1) and oligodendrocyte (MBP) markers.
In fact 74% of glioblastomas sequenced harbored aberrations in all three pathways, thus confirming that alterations in these three pathways are a core requirement of glioblastoma formation.
H-RasV12-shp53 and shNF1-shp53 lentivirus induced glioblastomas have grossly similar histological and morphological characteristics.
Characterization of gliomas induced by injection of H-RasV12-shp53 in the cortex of SynI-Cre mice
Gliomas induced by injection of H-RasV12-shp53 in the cortex of CamK2a-Cre mice
SynICre-tumors express high levels of progenitor/stem cell markers Nestin and Sox2
An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1

Verhaak RG et al, Cancer Cell, 2010
Gene expression data identify two GBM subtypes: Mesenchymal and Neural
Glioblastoma Multiforme

Post-op Recurrence
Characterization of Tumor Cells

Only 100 cells were injected into right hippocampus in NOD SCID mice.

4 out of 5 mice showed tumor formation (average $38.3 \pm 5.9$ days) so far, whereas control mice injected with normal neural stem cells did not.

Only 10 cells injected into right hippocampus in NOD SCID mice can also caused tumors.
CANCER STEM CELLS:

Cancer cells (found within tumors) that possess characteristics associated with normal stem cells:

Ability to self renew and the ability to give rise to all cell types found in a particular cancer sample.
Block replication of tumor stem cells

Neurosphere like Structure in EGF+ FGF+Heparin containing medium
005 cells were cultured in DMEM/F12/10%FBS for 5 days and fixed with 4%PFA.

005 cells differentiate into **astrocytes** (GFAP) and **neurons** (Tuj1)
High-resolution large-scale mosaic image of Ras-sip53 tumor (two weeks post-injection)
Confocal microscopy analysis of brain sections 5 days after injection of H-RasV12-shp53 virus in the cortex of GFAP-Cre mouse.
High-resolution large-scale mosaic imaging of tumor brain and microenvironment
Infected astrocytes switched to NSC media supplemented with FGF-2

A

White field | S-100β | GFAP | Overlay | GFAP/CRE/DAPI | Nestin/Sox2/Dapi

B

White field | RFP | GFP

Infected astrocytes switched to NSC media supplemented with FGF-2

C

H&E | GFAP | Nestin | Tuj1

D

GFAP/CRE/DAPI | Nestin/Sox2/Dapi

E

i

F

GFP | Nestin | Sox2 | Overall
Transduced Synl-Cre derived neurons reprogram to a progenitor/stem cell state
IPS and Cancer Stem Cell

**Normal Cell**
- Limited lifespan
- Differentiated

**Glial Cell**
- C-Myc
- Klf4
- Oct4
- Sox2
- Self-renewing
- Undifferentiated

**Glial, Neuronal, NSCs Cells**
- Ras
- or
- Nf1-/-

**siP53**

**Malignant Glioma**
- Sox2+, Nanog+cMyc+

**iPS cell**

**p53-/- Increased efficiency**

**Neurons**

**Glia**

**Oligodendrinocyte**
GBM is a Vascular Rich Tumor

• GBM (Grade IV) is a vascular rich tumor.

• VEGF is produced by tumor cells and high concentration of circulating VEGF is related to poor prognosis.

• In GBM but not in anaplastic glioma (Grade III), vascular endothelial proliferation and mitotic figures are seen.

(Wen PY and Kesari S. NEJM 2008)
Bevacizumab Therapy for GBM

Bevacizumab (Avastin): humanized anti-hVEGF MAb

- In a phase II trial of patients with recurrent grade III or IV (GBM) glioma patients, Bevacizumab and Irinotecan therapy showed response rate of 60%. But this effect is transient in most patients.

**Tumor vessels of Glioblastoma Mouse Model**

*Confocal microscopy: Immunofluorescence for von Willebrand Factor (vWF)*

- Many blood vessels in the GFP-positive tumor
- Blood vessels are lined with endothelial cells (Ecs)

<table>
<thead>
<tr>
<th>DAPI</th>
<th>GFP</th>
<th>vWF</th>
<th>Merge</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image]</td>
<td>![Image]</td>
<td>![Image]</td>
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<td>![Image]</td>
<td>![Image]</td>
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</tr>
</tbody>
</table>

Upper: 63X  
Lower: +3X Zoom
GFP+ Endothelial Cells (ECs) in the Tumor

Confocal microscopy: IF for vWF

DAPI  GFP  vWF  Merge

Upper: 63x  Lower: +3X Zoom
GFP+ ECs in the Mouse GBM

Tomo-Ras- and Tomo-Akt-induced mouse GBM

Animation of z-stack assay
GFP⁺ Endothelial Cells (ECs) in the Tumor

Confocal microscopy

CD31 (PECAM)

CD34

CD144 (VE-Cadherin)

63x +3x Zoom
Expression of VEGF-R2 in GFP⁺ ECs

Confocal microscopy

DAPI  |  GFP  |  vWF  |  VEGF-R2  |  Merge

Regular EC

GFP⁺ TDEC

GFP⁺ ECs: VEGF-R2 (-)
Cumulated survival rate

Days after vector injection

- --- 0.5%CMC (vehicle)
- AG28262 (100 mg/kg/d)

p=0.3688
(Logrank test)

TDEC⁺ vessels [%]

- Deep Area
- Border

Vehicle control
AG28262

* * ** NS
Figure legend: Endothelial differentiation of human GBM cells. (A) TDECs in a human GBM xenograft tumor. GBM sphere cells established from human GBM were transduced with lenti-GFP vectors and transplanted into NOD-SCID mice brain. Tumors were developed about 4 months after transplantation, and examined by immunofluorescence assay. Some vascular ECs were expressing not only vWF (red) but also tumor marker GFP (green) and human Nestin (white), strongly suggesting that the human GBM cells transdifferentiated into ECs. (B) Immunofluorescence assay of a human GBM sample. A representative result of tumor samples of human GBM patients. There were many vascular Ecs (vWF+, green) in the tumor. The tumor cells surrounding ECs were expressing EGFR, and vWF+ ECs were also expressing EGFR, suggesting the possible existence of TDECs in the human GBM.
**Endothelial differentiation of Neural Stem Cells**

**Cell fusion-independent differentiation of neural stem cells to the endothelial lineage**

Andrew E. Wurmser¹, Kinichi Nakashima¹,², Robert G. Summers¹, Nicolas Toni¹, Kevin A. D’Amour¹, Dieter C. Lie¹ & Fred H. Gage¹

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²Department of Cell Fate Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, 2-2-1 Honjo, Kumamoto 860-0811, Japan

**A** Coculture of GFP-labeled mNSCs and hECs

Mouse NSC (GFP+)

Human EC (GFP−)

2-5 days

Mouse NSC-derived EC (GFP+)

GFP+ cells expressed EC antigen (CD146)

**B** Coculture of GFP-labeled mNSCs and fixed hECs

• GFP+ cells bound with lectin (EC character)

**C** Transplantation of GFP+ NSCs into embryonic mouse telencephalon

GFP+ cells bound to lectin and showed EC-like morphology.

NSC could differentiate to EC in a fusion-independent mechanism.
Cancer Cell Plasticity

**Cell intrinsic**

Direct reprogramming by oncogenic Ras and Myc
Irene Iksichenkova, Jan Znir, Ute M. Moll, Alice Nemajerova, and Oleksandr Petrenko

Genetically or epigenetically defined reprogramming is a hallmark of cancer cells. However, a causal association between genome reprogramming and cancer has not yet been conclusively established, particularly, little is known about the mechanisms that underlie oncogenesis or whether it takes place in a broad range of cancers. Moreover, the concept of plasticity itself has been challenged, as cells in cancers with oncogene mutations or expression patterns that are not all cells in the normal tissue. In this study, we sought to address this question.

**Cell extrinsic**

Intestinal Tumorigenesis Initiated by Dedifferentiation and Acquisition of Stem-Cell-like Properties

Poised Chromatin at the ZEB1 Promoter Enables Breast Cancer Cell Plasticity and Enhances Tumorigenicity
Christelle L. Chauvet,1,2 Nemanja G. Mazarakis,1,2 Tony Lee,1,2 George Bell,1,2 Colbre. G. Klarel,4,5 Ferenc Reinhart,4,6 Ana C. Delbro,4,5,6 Richard A. Young,1,2,7,8 and Robert A. Weinberg1,2,7,8

Glioma tumorspheres

**LETTER**

Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation
Jennifer Lenshagen,1,2,3 Judith Kohlbrenner,1,3,4 Marcel Beno,1,2,3 Tobias Hufn,1,2,3 Meri Bogova,1,2,3 Maria Cervi,1,2,3,4,5,6 Natascha Lenz,1,2,3,4,5,6 Thomas Wolfs,1,2,3,4,5,6 Michael Hühn,1,2,3,4,5,6 Thomas Töllner1,2,3,4,5,6

Wnt activity defines colon cancer stem cells and is regulated by the microenvironment
Louis Vermeulen,1,2 Felipe De Sousa E. Neto,1,2,3,4 Maarten van der Heijden,1,2,3 Kate Cameron,1,2,4 Joan H. de Jong,1,4 Tijanna Borovkova,1,2,3,4,5 Jeroen R. Timmerman,1,2,3,4,5 Mathilde Tedeschi,1,2,3,4,5 Christian Nicke,1,2,3,4,5,6 Hans Rodermond,1,4,5,6 Martin B. Sprick,1,2,3,4,5,6 Kristel Kemper,1,2,3,4,5,6,7 Dick D. Biebel,1,2,3,4,5,6 Giorgio Staali1,2,3,4,5,6 and Jan Paul Medema1,2,3,4,5,6

Rajagopal et al ( Dedifferentiation airway)
Stochastic Vs Deterministic

Cellular states are not fixed, but still rigid to prevail an order:

- Dedifferentiation
- Waddington`s Landscape
- Reprogramming
- Transdifferentiation
New Therapeutic Approaches for Malignant Gliomas

Our Findings:

1. Gliomas can originate by dedifferentiation/reprogramming of astrocytes, neurons, oligodendrocytes and NSCs.

2. Every tumor cell in the glioma is capable of continuous proliferation and differentiation into all the CNS lineages found in the tumor.

3. As few as 10 glioma cells can induce GBMs in mice, with patho-physiology and molecular signatures in large measure similar to human GBMs.

4. Gliomas exhibit high expression of a number of “stem cell/self renewal” genes.

Possible Therapeutics: Inhibit expression of seminal genes involved in self-renewal, e.g. Bmi-1
Polycomb group (PcG) genes are epigenetic gene silencers that preserve transcription patterns to maintain cell identity, a function clearly compatible with a role in self-renewal.

Bmi1, a member of the polycomb family, has a profound effect on NSCs, reducing forebrain SVZ neurosphere frequency by 80% at 30 days after birth.
**Direct targeting of Bmi-1 by miR-128**

**miR-128 tumor suppression in a mouse glioma model**
1. Gliomas show constitutive activation of NFkB activity, leading to expression of many NFkB inducible target genes.

2. Loss of IkB kinase (IKK2) compromises the “stemness” of glioma cells, leading to differentiation.

Possible Therapeutics: Selective inhibition of NFkB activity
NFκB activation in HRas-shp53 induced glioma tumors

NFκB canonical (or classical) pathway

RNAseq analysis (150 NFκB target genes)
NF-kB target genes are upregulated in Ras-shp53 tumors (qPCR validation)

IkBa

PLAU

mIL-6

TNFa

Serpine-1

CCL-2

NBT – n=3
Ras-shp53 – n=5
Does NFκB pathway play a role in tumor cell plasticity?
Silencing IKK2 in NF53-10 tumor cell line

- **Cytometer**
  - *593/40 561*
  - *10^5 to 10^6*

- **Graphs**
  - O.D.
  - % survival
  - Days
  - Hours

- **Images**
  - IKK2
  - tubulin
  - NF53-10
  - NF53-10-shcMET
  - NF53-10-shIKK2
  - NF53-10-shcMET

- **Legend**
  - shIKK2
  - shcMET
  - shIRR
  - control
  - NF53-10-shlRR
  - NF53-10-shIKK2
Floxed IKK2 transgenic mice / CRE-ErT2 system

Infection of primary cortical f/f IKK2 astrocytes with the above virus

Astro1  Astro2  Astro3
(-)  (+)  (-)  (+)  (-)  (+)  4-OH tamoxifen

47k2

Tubulin
Twenty days after transplantation of infected astrocytes (Astro1f/flkk2R53iresCreERT2) one group of mice was injected with 4-OH tamoxifen (4-OHTMX) for 5 consecutive days i.p. and the other group received vehicle as control.
Depletion of Ikk2 in transformed astrocytes blocks tumor formation of tumorspheres.

Astrocytes infected with Hras-shp53iresCREerT2 were treated with 4OHTMX and switched to NSC media.
Selective inhibition of NFκB activation—NBD peptide

- Activation of NFκB requires the activity of IκB kinase (IKK) complex containing IKK1 and IKK2 and the regulatory protein NF-κB essential modifier (NEMO).
- The peptide corresponding to the NEMO-binding domain (NBD) of IKK1 or IKK2 specifically inhibit the induction of NF-κB activation without inhibiting basal NF-κB activity.

<table>
<thead>
<tr>
<th>Name</th>
<th>PTD</th>
<th>NEMO Binding Peptide</th>
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<tbody>
<tr>
<td>PTD–5</td>
<td>RRQRRRTSKLMKR</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>8K</td>
<td>KKKKKKKKK</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>6R</td>
<td>RRRRRRR</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>TAT</td>
<td>YGRKKRRQRRR</td>
<td>TALDWSWLQTE</td>
</tr>
<tr>
<td>Antp</td>
<td>RQIKIWFQNRRMKW</td>
<td>TALDWSWLQTE</td>
</tr>
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</table>
NBD peptide inhibits NFκB activation and prolongs mice survival

qPCR analysis – NFκB target genes

Entry of NBD peptide in the CNS

Ghosh et al, PNAS 2007, 104:18755
NBD peptide inhibits NFκB activation and prolongs mice survival

The 005 tumor cells were transplanted at day 0 and the treatment started at day 12. Mice received either saline or NBD (0.2 mg/day, i.p.) daily for 14 days.
Gene interaction network of differentially expressed genes
1. Osteopontin (OPN), also known as Spp1, is highly expressed and secreted by the tumors and dedifferentiated/reprogrammed cells.

2. Attempts to inhibit the action of OPN blocked the formation of tumorspheres and diminished their proliferating capacity.

Possible Therapeutics: Selective inhibition/neutralization of OPN, and as recently suggested by E. Holland’s group, the OPN receptor: CD44 (which is highly expressed in our mouse tumors and cell lines)
1. Both mouse and human GBMs can transdifferentiate into endothelial (and pericytes) cells, forming functional blood vessels.

2. TDECs are enriched in FGFR, offering the possibility of a dual inhibitor of both VEGFR & FGFR to block angiogenesis & transdifferentiation.

Possible Therapeutics: Use of dual inhibitors, Brivnib, JNJ 42756493, C-Met inhibitor
Effect of Neutralization Ab for VEGF on Tube Formation

Matrigel assay
Day 5 cells
(20 hr after Matrigel culture)

<table>
<thead>
<tr>
<th>DFS/DFO(+)</th>
<th>DFO (-)</th>
<th>DFO (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-VEGF (-)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-VEGF (+)</td>
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</table>

• Tube formation of 005-derived ECs was VEGF independent.
Brivanib, A Dual Inhibitor of VEGF and FGF RTKs

- Inhibits phosphorylation of FGFRs and VEGFRs at sub-µM concentration.
- Inhibits angiogenesis and tumor growth in mouse xenograft models of lung and colon cancers, and hepatocellular carcinoma (HCC), etc.
- Under phase III clinical trial for HCC patients.

Clin Cancer Res, 2008
**Effect of Brivanib on Tube Formation**

**Brivanib**: selective dual inhibitor of VEGF-Rs and FGF-Rs

Tube formation assay
005 cells
EGM/DFO (-)

<table>
<thead>
<tr>
<th>Control (No inhibitor)</th>
<th>Brivanib [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
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</table>

**Bright**

**GFP**
Treatment of GBM-harboring mice by Brivanib

GFAP-Cre mice injected with Tomo-Ras-sip53 LV

Mouse brains

Brain weight

- Brain size: Control > Brivanib

Smaller tumors in brivanib-treated mice was suggested.
Decrease of Tumor Vessels by Brivanib treatment in mouse GBM

GFAP-Cre mice injected with Tomo-Ras-sip53 LV

Control Brivanib

Vessel Density [μm²]

Control Brivanib

*p<0.01*

Mann-Whitney U-test
GFAP-Cre mice injected with Tomo-Ras-sip53 LV

Brivanib inhibited TDEC formation in the mouse GBM model

Decrease of TDECs in Brivanib-treated GBM

- Brivanib inhibited TDEC formation in the mouse GBM model

* Mann-Whitney U-test
Brivanib inhibited tumor growth in GBM mouse

GFAP-Cre mice injected with Tomo-Ras-sip53 LV

- Brivanib inhibited tumor growth
Treatment of GBM-harboring mice by Brivanib

GFAP-Cre mice injected with Tomo-Ras-sip53 LV

Survival curve (Kaplan-Meier)

Treatment

- Vehicle Control
- Brivanib 100mg/kg

Cumulative Survival Rate vs. Days after Injection

$p=0.927$ (Logrank test)
Increased invasiveness of Brivanib-treated GBM

GFAP-Cre mice injected with Tomo-Ras-sip53 LV

- Brivanib-treated tumors were more invasive than untreated tumor.
- Intraventricular invasion induced significant hydrocephalus.
U.S. death rate* by cause, 1950 and 2002:

Heart diseases: 586.8
  - 1950: 240.1
  - 2002: 240.1
Cerebrovascular diseases: 180.7
  - 1950: 180.7
  - 2002: 56.0
Pneumonia/influenza: 48.1
  - 1950: 48.1
  - 2002: 22.5
Cancer: 193.9
  - 1950: 193.9
  - 2002: 193.4

*Rates are per 100,000 and are age-adjusted to the 2000 U.S. standard population.

Cancer Deaths Drop From 1991-2009

• Cancer death rates fell 24% among men and 16% among women, and 20% Overall

• This will translate to 1.2 M cancer deaths that have been prevented since 1991

  - all four major cancers: lung, breast, prostate and colorectal
    – larger and older US population

Prevention

Early Diagnosis

New Treatments

ACS 2013
Molecular Targeting of Therapy: Proof of Concept

All-trans Retinoic Acid: APL (PML-RAR)
Tratuzumab/Herceptin: Breast Cancer (HER2)
Imatinib/Gleevec: CML/GIST/HES (BCR-ABL)
Gefitinib/Iressa: NSCLC (EGFR)
## Cancer drugs that act against tyrosine kinases

<table>
<thead>
<tr>
<th><strong>DRUG</strong></th>
<th><strong>CANCER</strong></th>
<th><strong>TARGET</strong></th>
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<tbody>
<tr>
<td><strong>Small molecule drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleevec™ (imatinib)</td>
<td>leukemia (CML)</td>
<td>Bcr-Abl tyrosine kinase</td>
</tr>
<tr>
<td>Iressa™ (gefitinib)</td>
<td>lung cancer</td>
<td>EGF receptor TK</td>
</tr>
<tr>
<td>Tarceva™ (erlotinib)</td>
<td>lung cancer</td>
<td>EGF receptor TK</td>
</tr>
<tr>
<td>Sutent™ (sunitinib)</td>
<td>GI stromal tumor/RCC</td>
<td>Kit receptor TK</td>
</tr>
<tr>
<td>Sprycel™ (dasatinib)</td>
<td>leukemia (CML)</td>
<td>Bcr-Abl tyrosine kinase</td>
</tr>
<tr>
<td>Tykerb™ (lapatinib)</td>
<td>breast cancer</td>
<td>ErbB2 RTK</td>
</tr>
<tr>
<td>Many in trials</td>
<td>several types/AML</td>
<td>angiogenesis/Flt3 RTKs</td>
</tr>
</tbody>
</table>

| **Monoclonal antibody drugs** | | |
| Herceptin™ (trastuzumab) | breast cancer | ErbB2 RTK |
| Erbitux™ (cetuximab) | breast/renal cancer | EGF receptor TK |
| Avastin™ (cevacizumab) | colon cancer | VEGF |

> 70 protein kinase inhibitors are in cancer clinical trials, including several directed against serine/threonine kinases implicated in cancer. The Raf serine/threonine kinase inhibitor sorafenib (Nexavar™) has recently been approved for treatment of renal carcinoma. Rapamycin, an mTOR kinase inhibitor, and analogues (e.g. temsirolimus and everolimus) are also in clinical trials for several cancers.
Within Next Two Decades Cancer Will Become a Chronic Disease

Treatments will be a Combination of Therapies that Include Standard Practices of Surgery, Radiation, Chemotherapy, Immunotherapy & Molecularly Targeted Therapies
Cancer as “Overlaid with mystification...a triumphant mutation...charged with fantasy of inescapable fatality...a scandalous subject for poetry...”

Susan Sontag, 1978 Illness As A Metaphor

2014:

The mystification is not a black box, the triumphant mutation has been exposed and Cancer is in retreat. We see new ways by which to confront that inescapable fatality.... And there is every reason for poetry.
FOR THE 15 MILLION CANCER SURVIVORS IN THE USA
Many Thanks For Listening

50th Anniversary of the Salk Institute
Summary

1) Lentiviral vectors can be used to generate mouse models of human cancer in a cell specific manner

2) GBMs originate via reprogramming of differentiated cells

3) Glial, neuron, neuro-progenitor cells or oligodendrocytes in various parts of the brain can give rise to GBMs

4) Both mouse and human GBM tumor cells can transdifferentiate to functional endothelial cells lining the blood vessels

5) This is a general method to make mouse models of human tumors (lung, pancreas, prostate)
Astrocytes transduced with LV-HRAS-shp53, switched to stem cell media and incubated for 5 days in the presence of neutralizing anti-OPN antibody. 

Blocking OPN significantly reduces the formation of neurospheres.
Neurospheres formation in the control wells

Impaired neurospheres formation when adding anti-OPN neutralizing antibody to the well
Astrocytes transduced with LV-HRAS-shp53 and shRNA targeting OPN (red)
Cultures were switched to stem cell media after shRNA transduction.

Silencing OPN reduces the formation of neurospheres