Personalized Hematology – Oncology OF WAKE FOREST

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Gene expression profiling for targeted cancer treatment

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Current landscape of cancer care

1. In the past 7 years, nearly 900 independent oncology practices in the United States have closed, been acquired by a hospital or merged with another entity, according to the Community Oncology Alliance. Several hundred more are struggling financially.

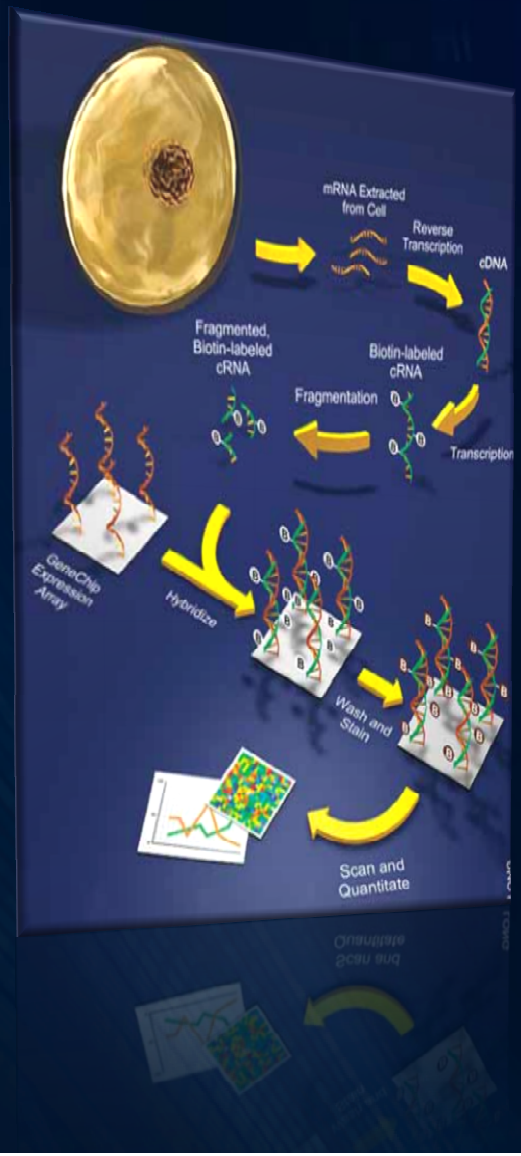
2. As expenses rise, reimbursements decline and regulatory burdens intensify, some experts suggest the trend will continue.

3. Others suggest independent providers who are willing **to adapt** to the evolving health care environment and **embrace creative strategies** can find ways to thrive.

HemOnc today, October 10, 2014

the research bench to the physician's tool for diagnosis and treatmen

Gene expression profiling –> experiment design



1. core biopsies from tumor and normal tissue preserved in RNA-later
2. RNA extraction and QC - clean, intact RNA will ensure the generation of high quality microarray d
3. synthesis of double stranded cDNA from the RNA sample using reverse transcriptase and an oligo
4. in vitro transcription (IVT) reaction that produces amplified amounts of biotin-labeled antisense m
5. cRNA fragmentation using heat and Mg+2 (this fragmentation reduces the cRNA to 25-200 bp fra
6. cRNA hybridization at 45 degrees Celsius for 18 hours
7. Staining the chip (U133 Plus 2.0) with a fluorescent molecule (streptavidin-phycoerythrin) that bir
8. series of washes and stains binds the biotin and provides an amplified flour that emits light when t
   is then scanned and the images processed using Affymetrix software, GeneChip Operating Softwa
9. MAS file types are generated: Experiment File *.EXP, Image Data File *.DAT, Cell Intensity File *.C
   Probe Array Results File *.CHP, Report File *.RPT, and MAGE-ML *.XML
10. Importing CEL file into Elsevier Pathway Studio software.

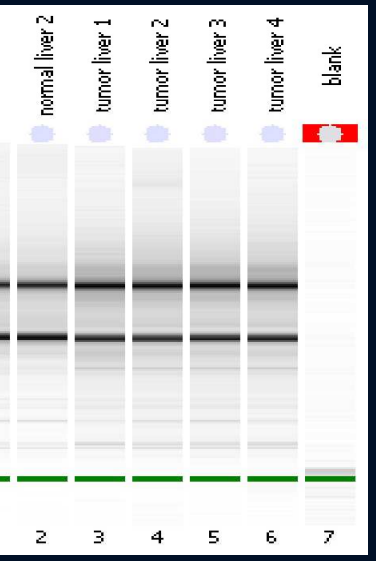
First patient

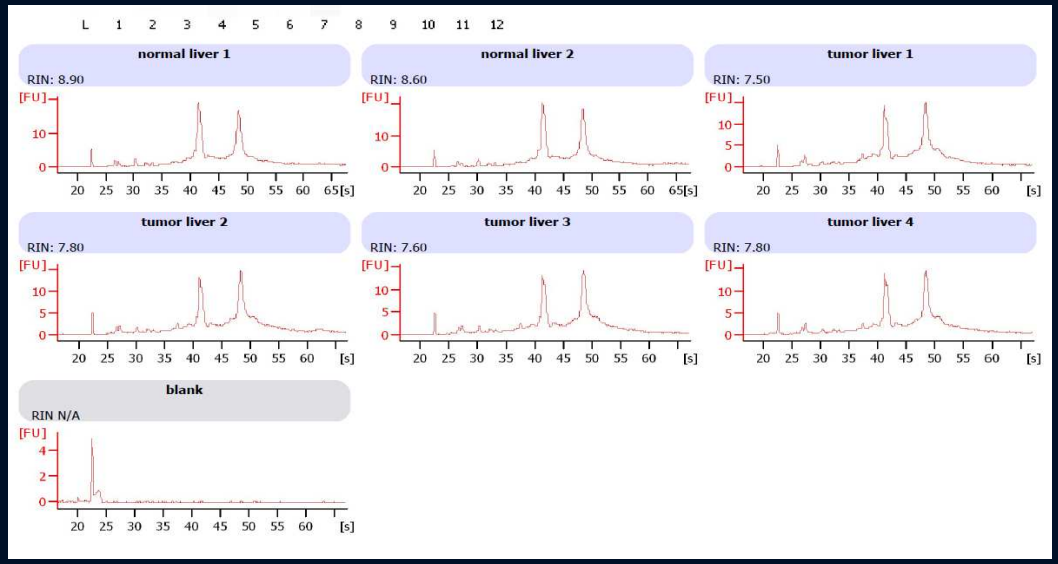
ear old Caucasian female diagnosed with moderate to poorly differentiated hepatocellular carcinoma with associated necr

t scan shows 9.0x7.2x5.7 cm right hepatic lobe mass

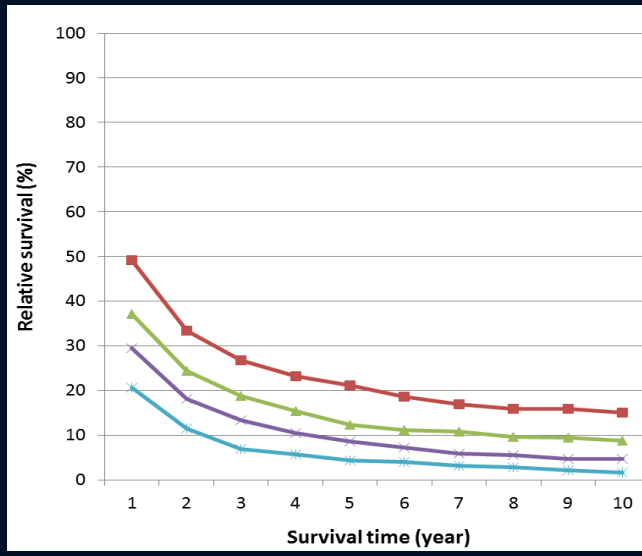
ction of hepatocellular carcinoma involving an ascending colon in the right lateral abdominal wall, segments 5 and 6 from and 11 benign lymph nodes

biopsies of liver tumor as well as some of the normal liver parenchymal cells were sent for gene expression profile ana



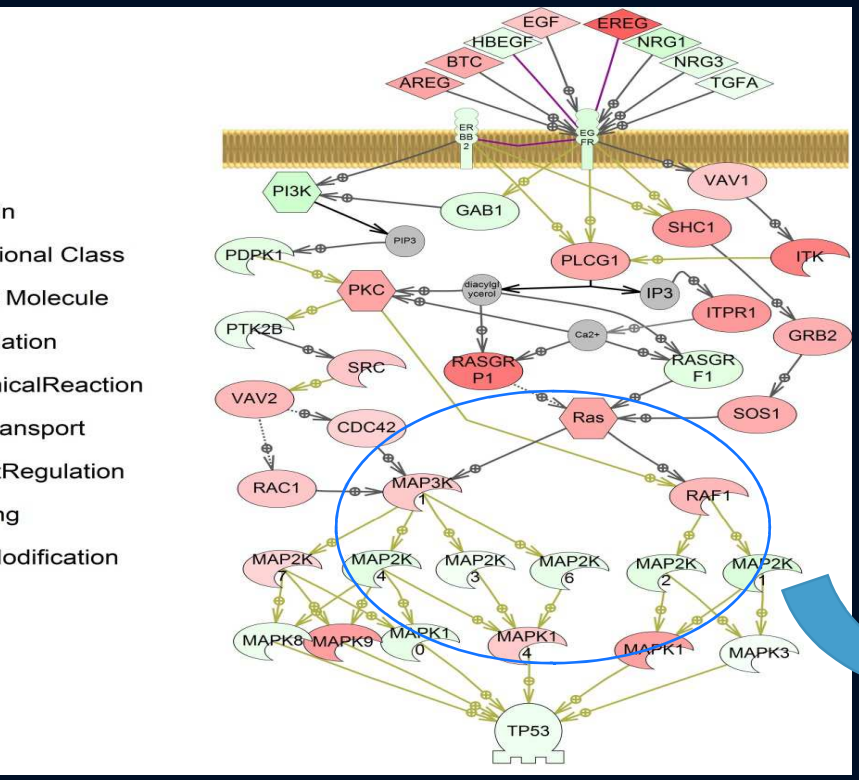


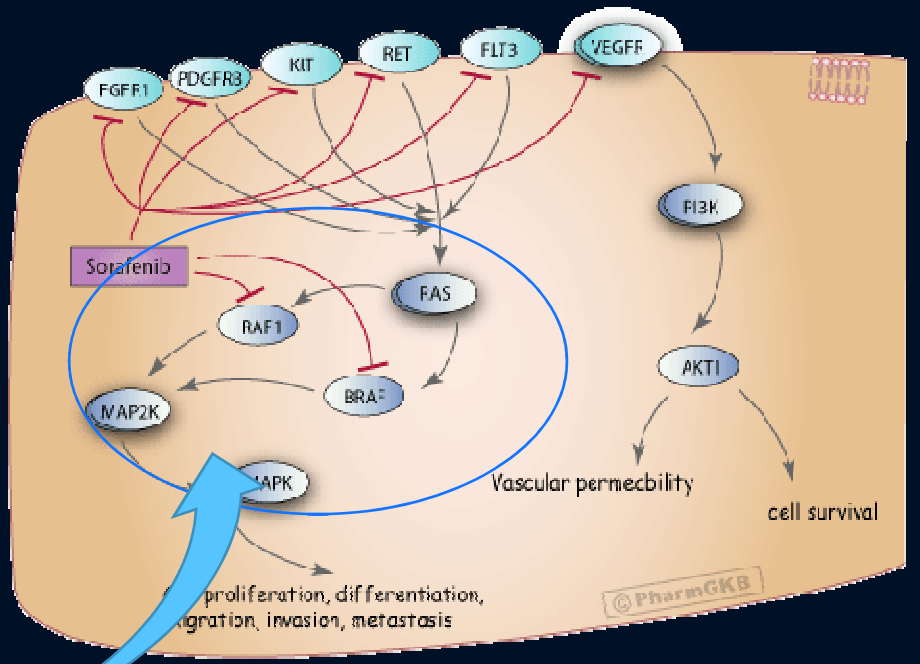
Liver cancer survival in women by survival tir

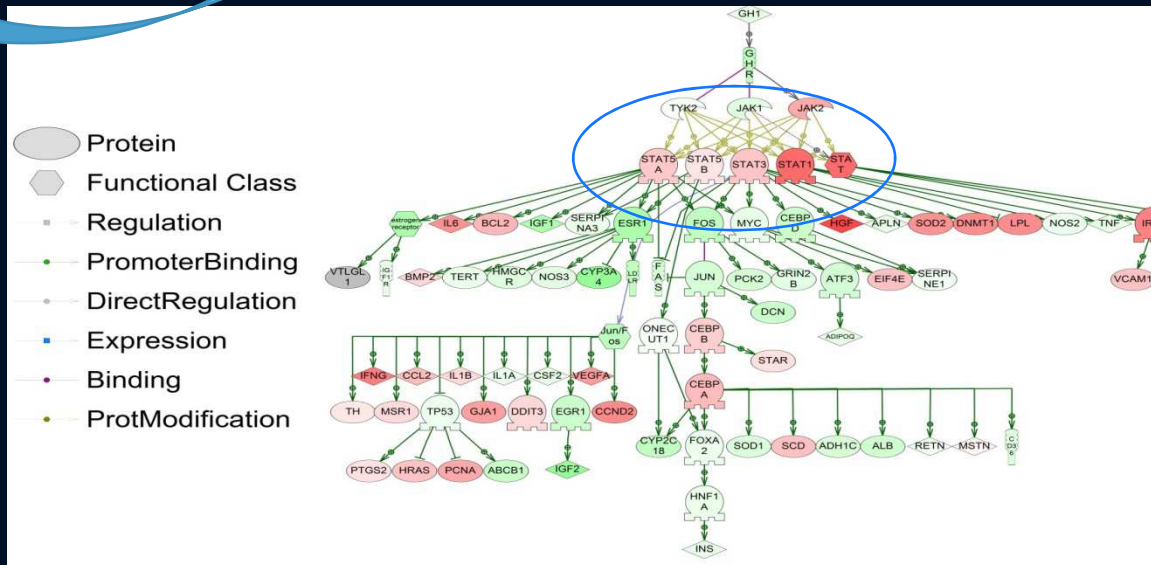


The figure shows survival rates for liver cancer by and age for the US male and female between 198

nt signal transduction pathways through which
tocellular carcinoma has proliferate is through
he Raf- Ras-MEK-Map kinase pathway.







athways within her hepatocellular carcinoma
JAK-STAT
SMAD – RUNX2
AKT1-FOXO1-IGFBP1
RUNT - VEGFA

t arranged to receive Sorafenib 400 mg twice a day at least six months

tion of residual disease with gene expression profile using mesenteric lymph node material from small bo

t referred to Virginia, Massey Cancer Center to look at being randomized in the clinical trial (ClinicalTrials.g
er: NCT01075113) looking at combinations of molecular targeted therapies inducing apoptosis (Vorinostat) in
dominant pathway, which were blocked by Sorafenib: EGFR–Ras–Raf– MEK–Map kinase–ERK pathway.

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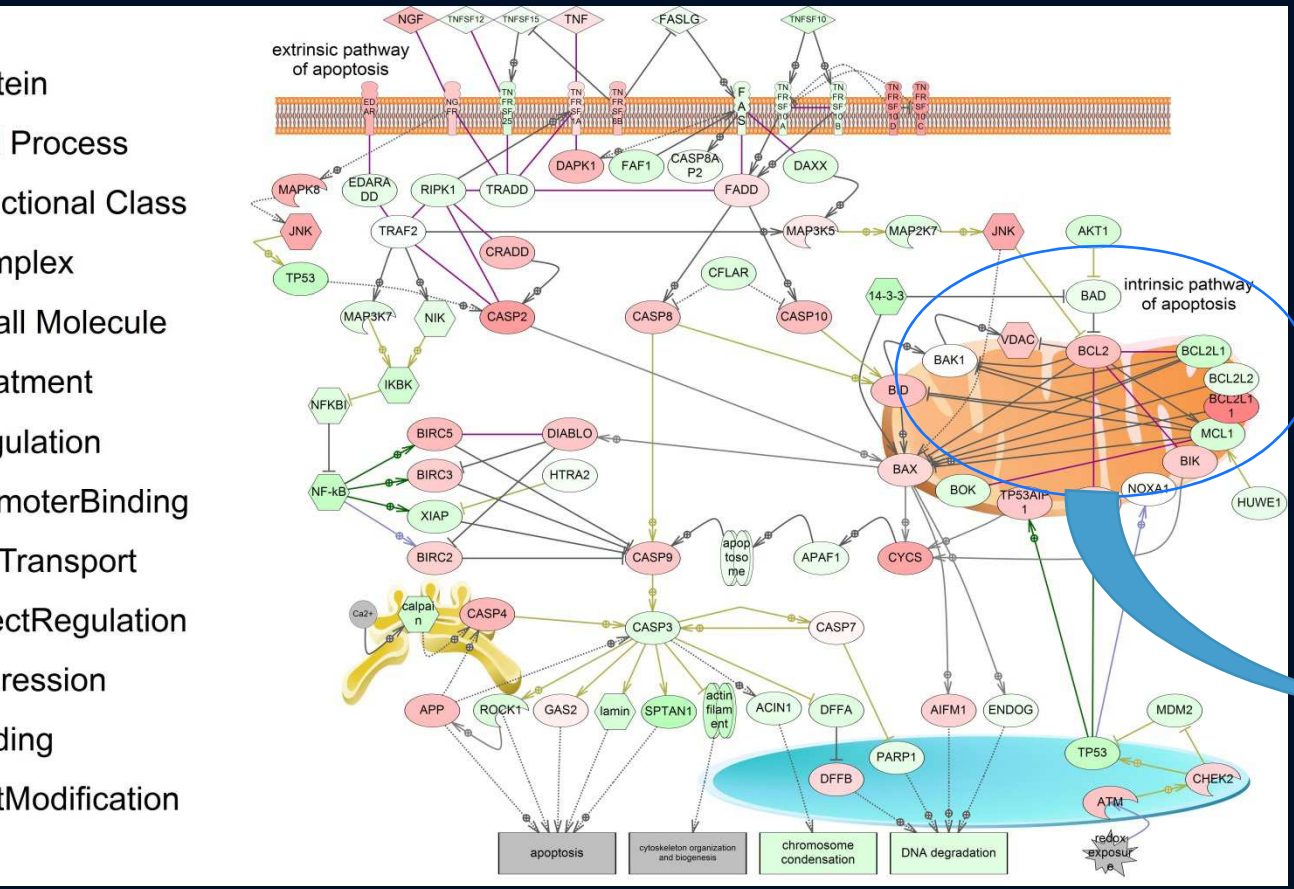
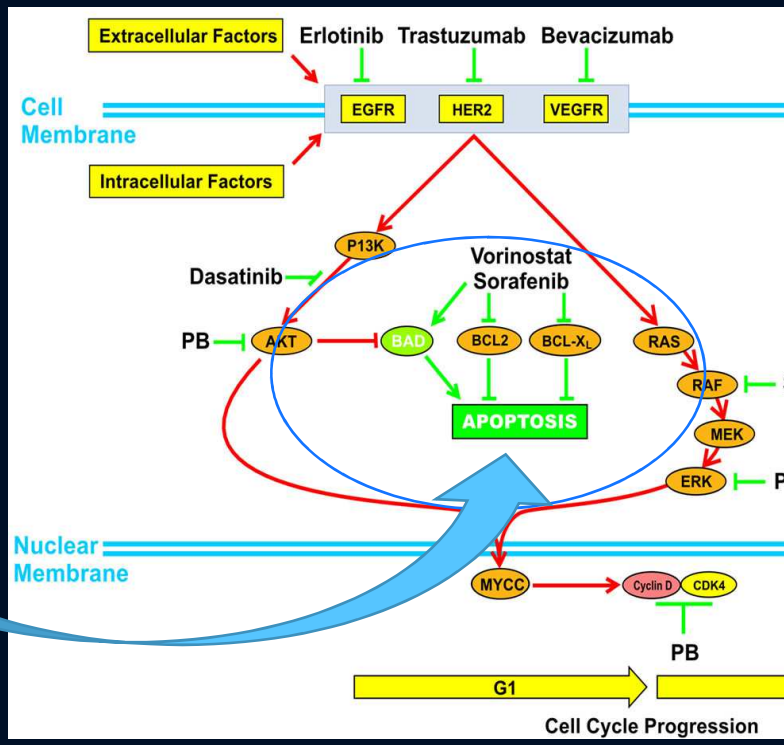
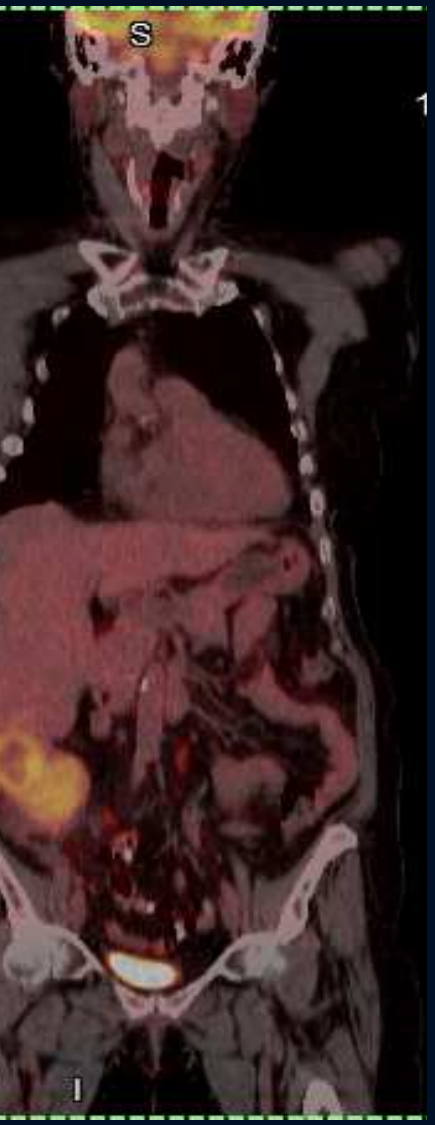


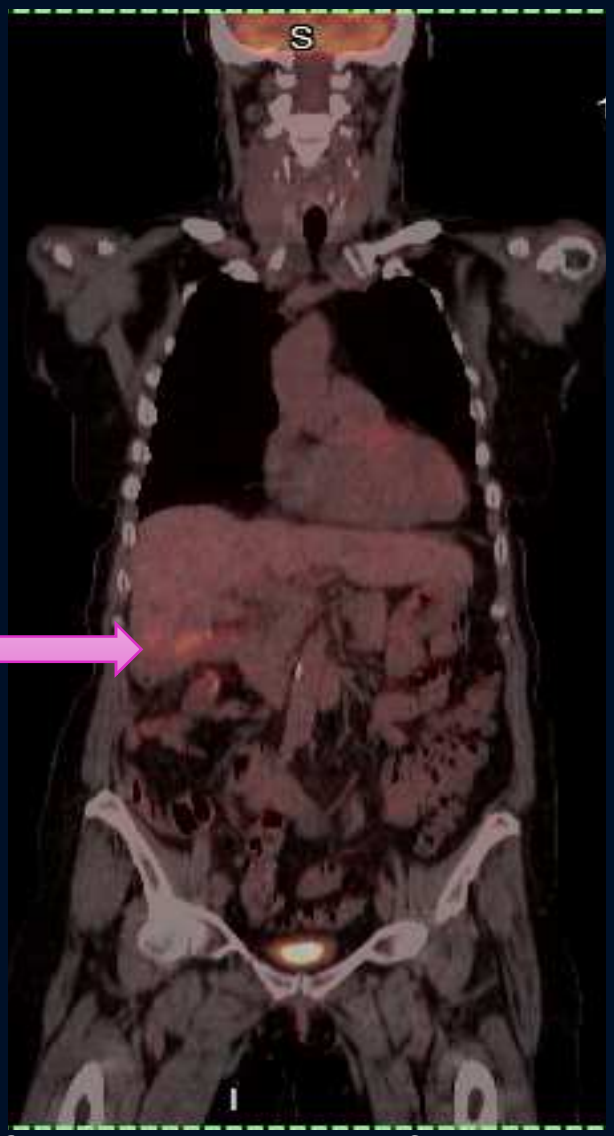


hase I trial is studying the side effects and best dose of vorinostat when given together with sorafenib tosylate in treating patients with advanced liver cancer. Sora
may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth or by blocking blood flow to the tumor. Giving sorafenib tosylate togeth
kill more tumor cells. – www.clinicaltrials.gov

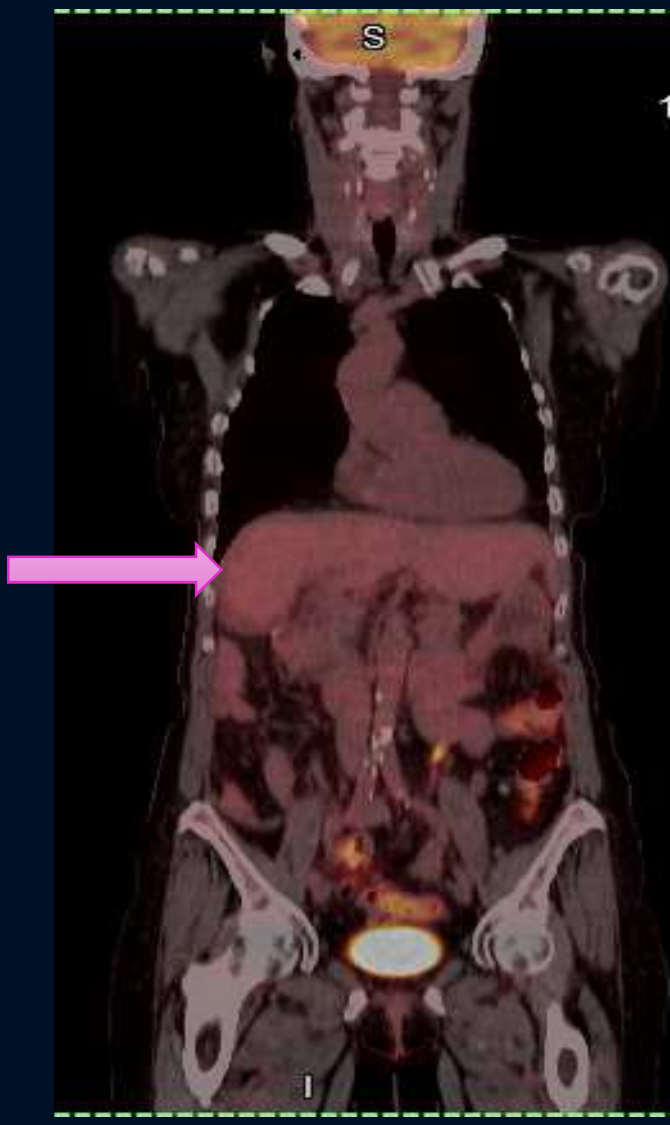
Before and after treatment



efore treatment
T/Scan-July, 2013



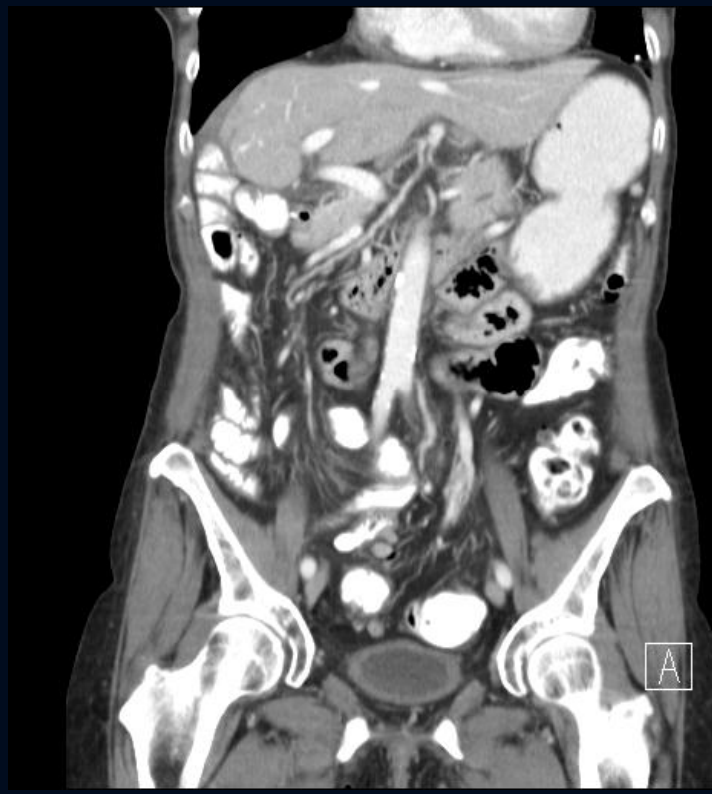
After resection and **Sorafenib** 400 mg twice a day (six months)-PET/Scan



After **NCT01075113 clinical trial Sorafenib plu**
Massey Cancer Center, Virginia, June 2014-P

First patient summary

- ✓ Continue the clinical trial
- ✓ Patient has outlived Overall Survival estimates based on standard of care treatment and continues to have normal quality of life with intermittent grade I hand–foot syndrome from her Sorafinib + Voronistat treatments on Clinical trial and to this date in time appears to be in clinical remission.



CT/Scan from 10-October-20

Second patient

ar old Caucasian female diagnosed in 2011 in Florida with stage I breast cancer, miss-labeled as ER+ /PR+ , and treated
taxel/Cyclophosphamine (4 cycles) followed by hormonal therapy

er recurrence in 2013 and diagnosed with stage IV breast cancer with mets in the right lung and her brain (diagnose mad
ractice for the first time after moving to North Carolina)

iagnosed (initial tumor block from Florida) as ER-/PR- and treated with radiation for the brain met and 2 cycles of
mycin/Cyclophosphamide (dose dense standard of care therapy, based on ASCO and NCCN guidelines) for the breast ca
mets

met responded to the radiation treatment but the lung met did not respond to AC chemotherapy and core biopsies were
rmed from the lung met

ment was switched to Gemcitabine (standard of care) until the gene expression profiling data was processed

expression profiling reveals:
ER -, PR-, Her2Neu- confirming pathology findings as a triple negative breast cancer metastasis
Cell invasion through: connective tissue growth factor (CTGF)->fibronectin 1 (FN1) ->integrin α-5 pathway
Cell cycle is activated through transcription factor activator E2F and viral oncogene MYBL2
Angiogenesis is up-regulated through VEGF

d on the tumor pathways , bevacizumab (recombinant human monoclonal antibody that blocks angiogenesis by inhibiting
FA) was added to her treatment to decrease blood vessel ingrowth to her tumor from angiogenesis

ated scan showed considerable decreasing on the lung met to the size that allowed it to be resected follow up by 2 more
vacizumab plus Gemcitabine

g the treatment, bone mets developed and she received 10 days of radiation as a palliative treatment

gn of disease and next scan is scheduled for November 2014

econd patient – tumor signaling pathway

ein
ctional Class
all Molecule
ulation
micalReaction
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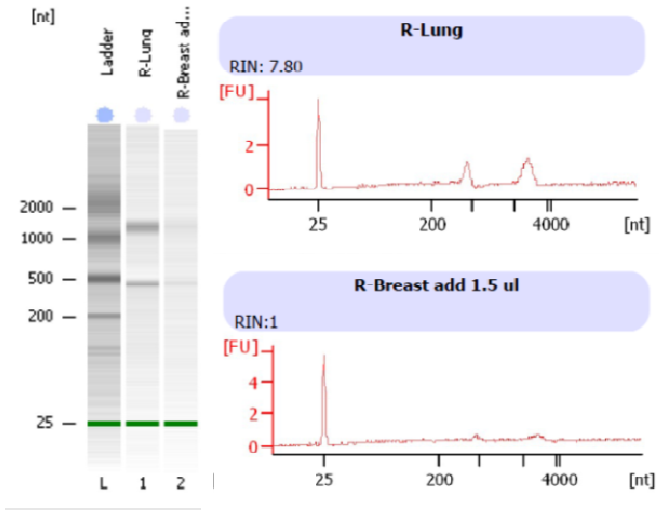
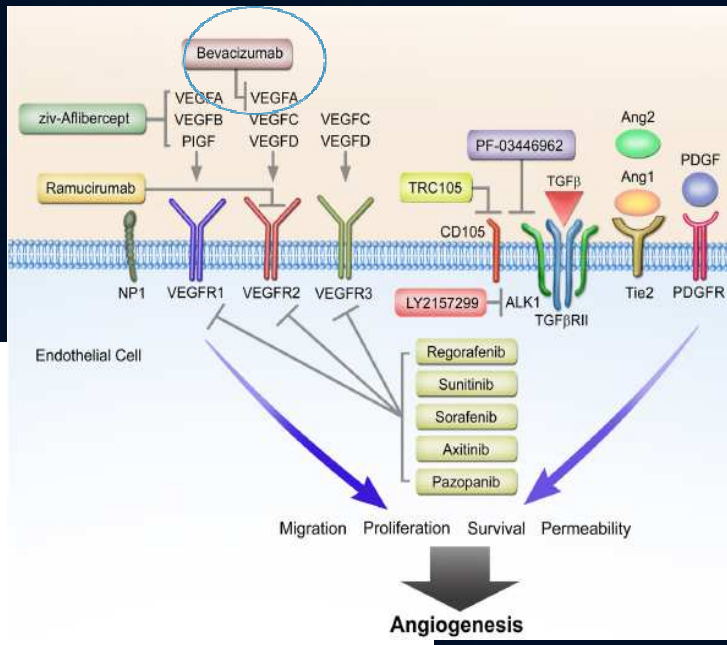
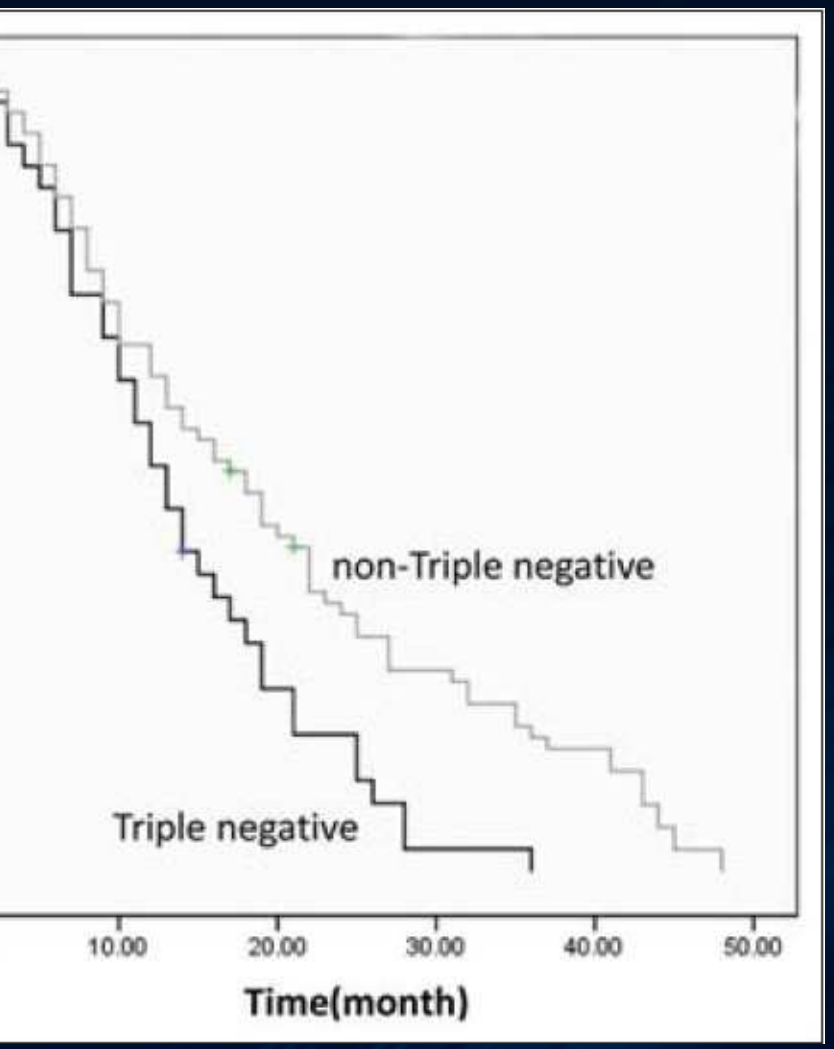
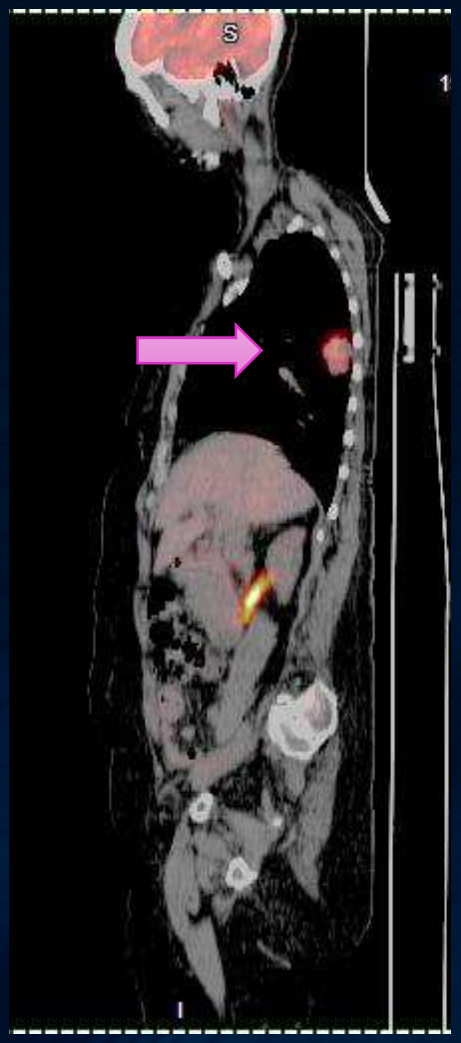
ling
Modification

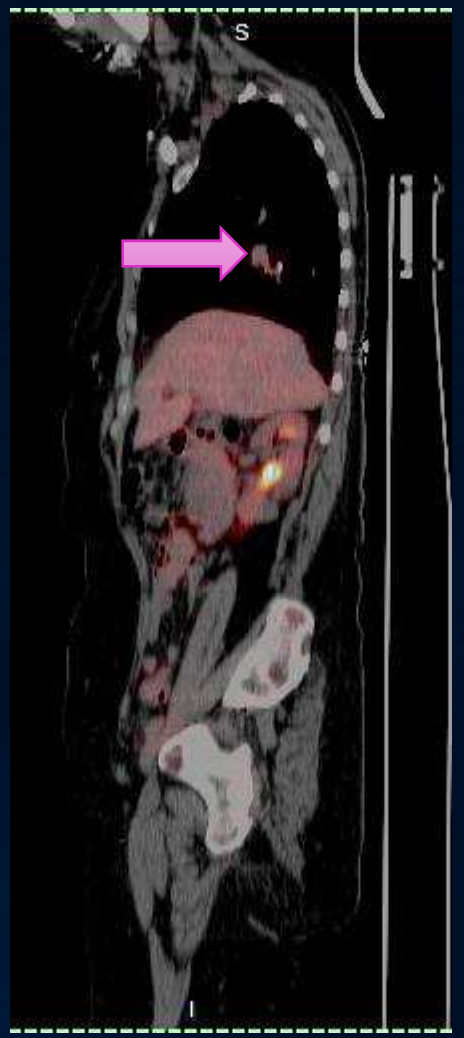




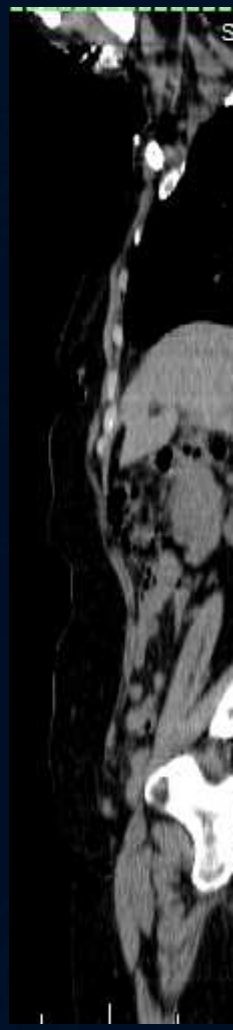




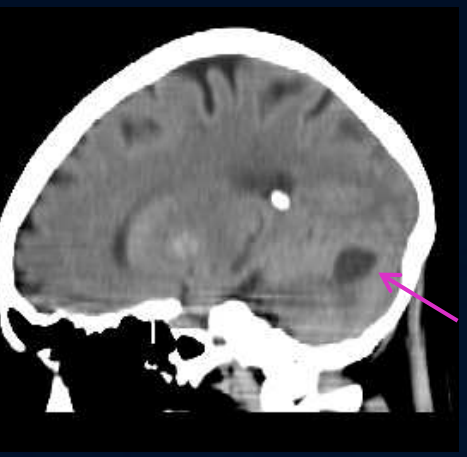
Lung met
before treatment
12 September 2013
PET/Scan



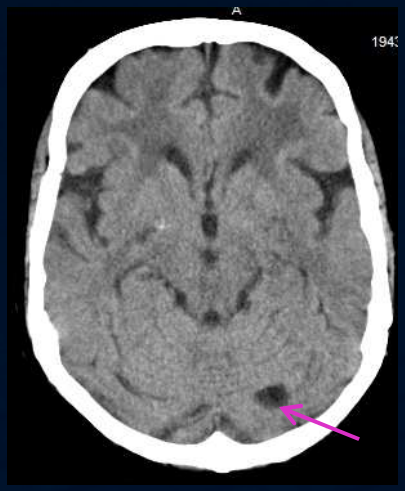
No lung met
after treatment
8 July 2014
PET/Scan



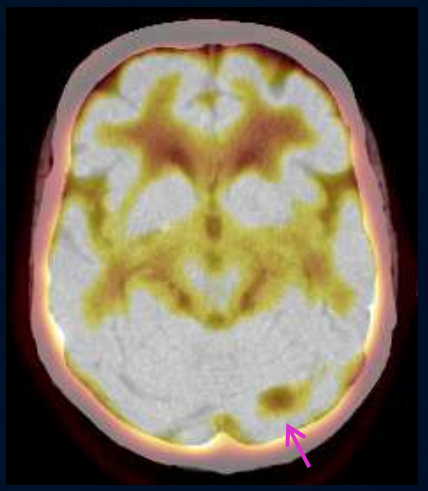
No lu
after tre
8 July
CT/



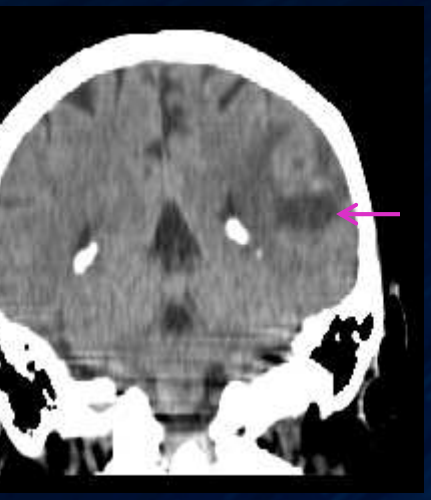
pt-2013: left **cerebellum** met
CT/Scan



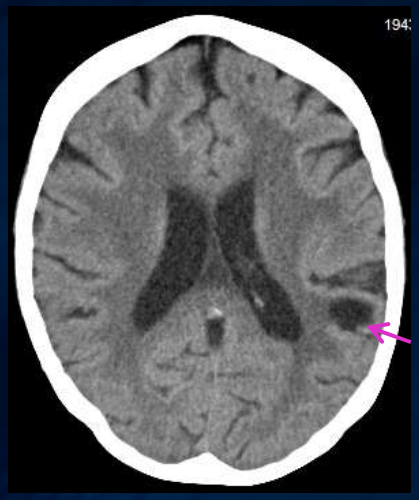
8-july-2014: left **cerebellum** no change in size
CT/Scan



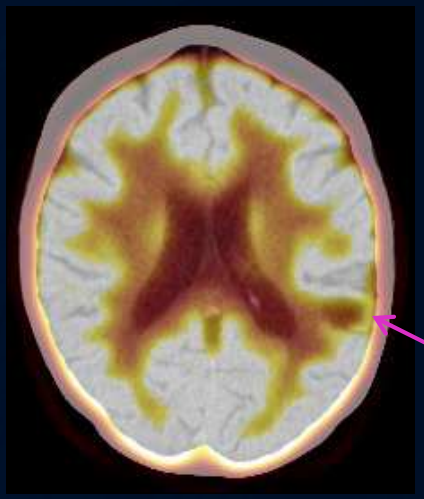
8-july-2014: left **cerebellum** no
hyper-metabolic activity PE



pt-2013: left **parietal** met
CT/Scan



8-july-2014: left **parietal** no change in size
CT/Scan



8-july-2014: **left parietal** no in
hyper-metabolic activity PE

Second patient summary

No clinical signs or symptoms of disease and next PET/CT scan is scheduled for November 2014

Patient has outlived Overall Survival estimates based on standard of care treatments and continues to have normal quality of life

Third patient

ear old Caucasian male diagnosed in 2009 with stage IV colon cancer

- Removal of sigmoid colon
- Radiofrequency ablation for two liver lesions
- Treated for surgical site infection
- Refused to have chemotherapy (adjuvant therapy) initially after the surgery

er recurrence in 2013 with multiple mets in the liver and lung; core biopsies were performed from the liver met for gene
ession profiling

nuary 2014 started standard of care modified FOLFOX6 regimen every 2 weeks with 5-FU CADD pump

- FOLFOX6 = 5-FU + Oxaliplatin + Leucovorin

expression profiling reveals:
tumor grows due to mitotic activation
FoxMP1 activation in the tumor ->FoxMP1 confers resistance to many drugs including chemo drugs
drug metabolization pathways are active -> bad outcome because tumor adapts to liver and liver's function is drug meta
angiogenesis is up-regulated through VEGF

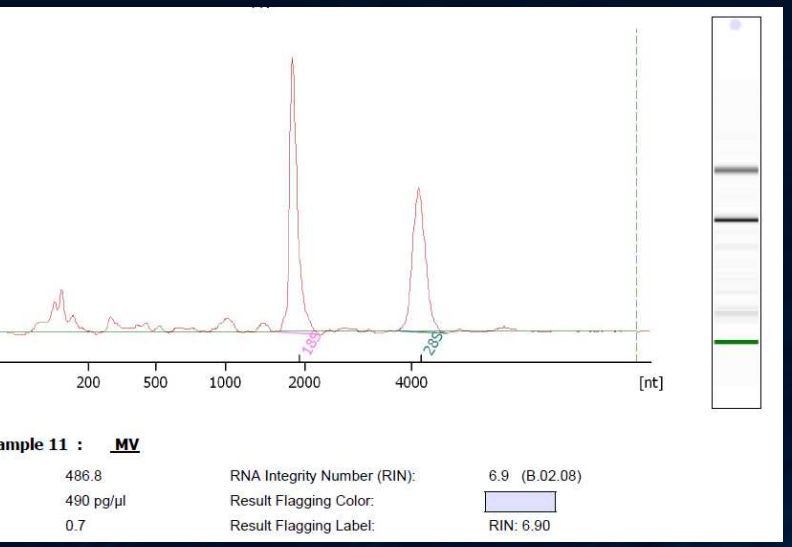
d on the tumor pathways

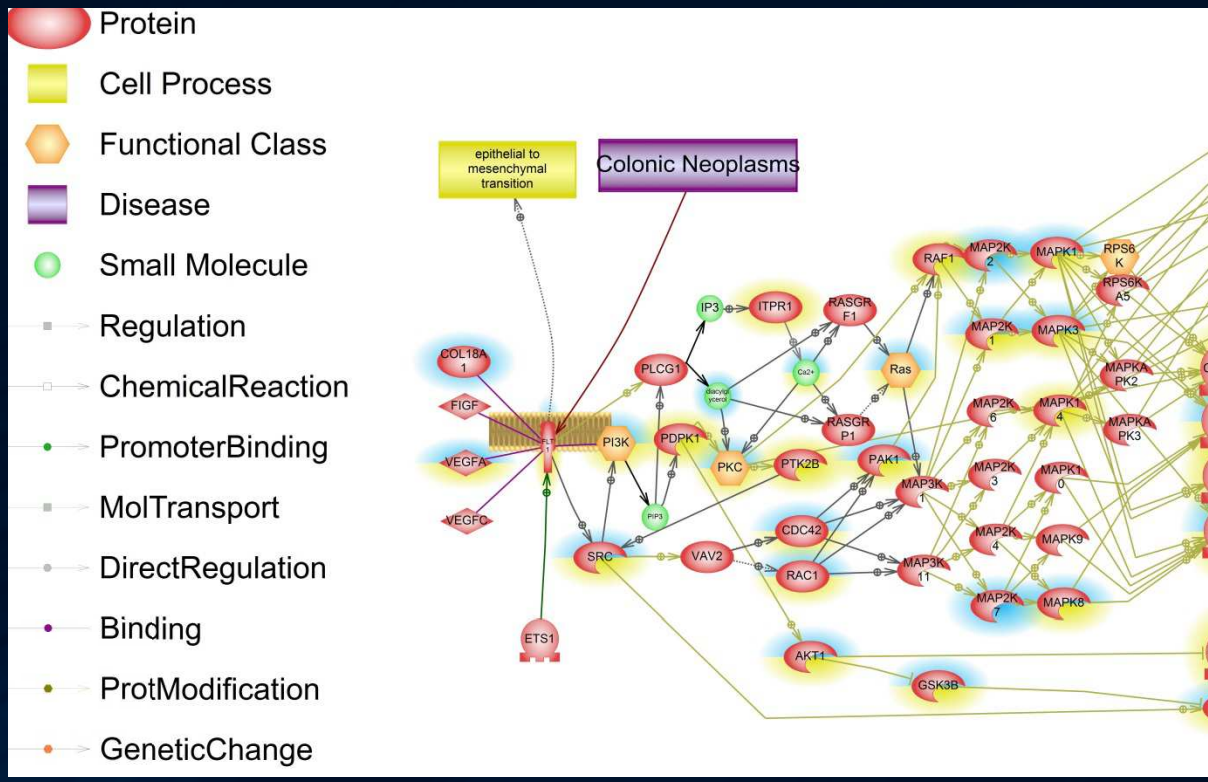
- Dexamethazone (blocks FoxMP1) was added to his original treatment (FOLFOX6) but without oxaliplatin to decrea resistance to chemo drugs; the other FoxMP1 inhibitors are not FDA approved for colon cancer
- Potential to use Ramucirumab – a VEGF Receptor 2 antagonist that specifically binds and blocks activation of VEG Receptor 2 and blocks binding of VEGFA receptor which binds VEGF-A, VEGF-C, and VEGF-D ligands
- September 2014, a pharma company announced use of Ramucirumab in patients with metastatic colorectal cancer benefit in overall survival

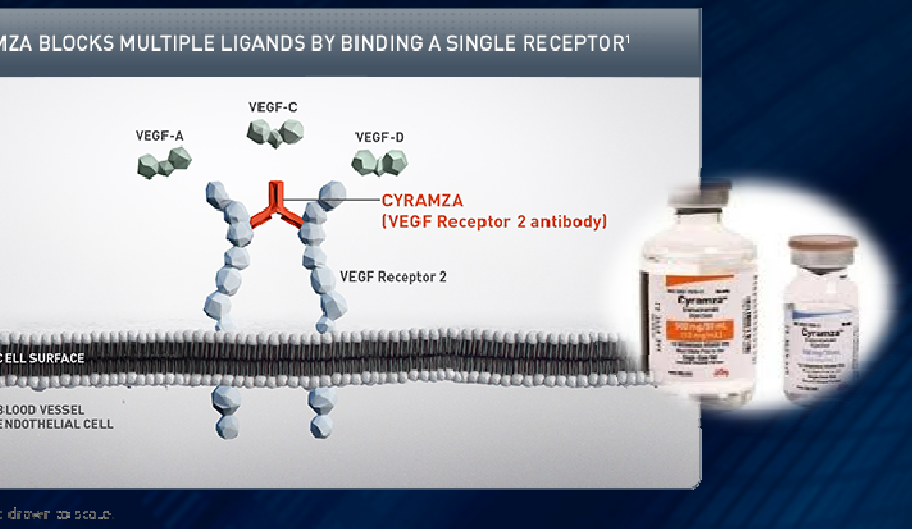
and July 2013 the patient was on hold from the toxicity of chemo treatment due to: typhilitis, pneumotosis of the colon (
olonic wall) and pulmonary embolism; started back on treatment in August 2014 but after 3 cycles decided to not have a
otherapy at that time.

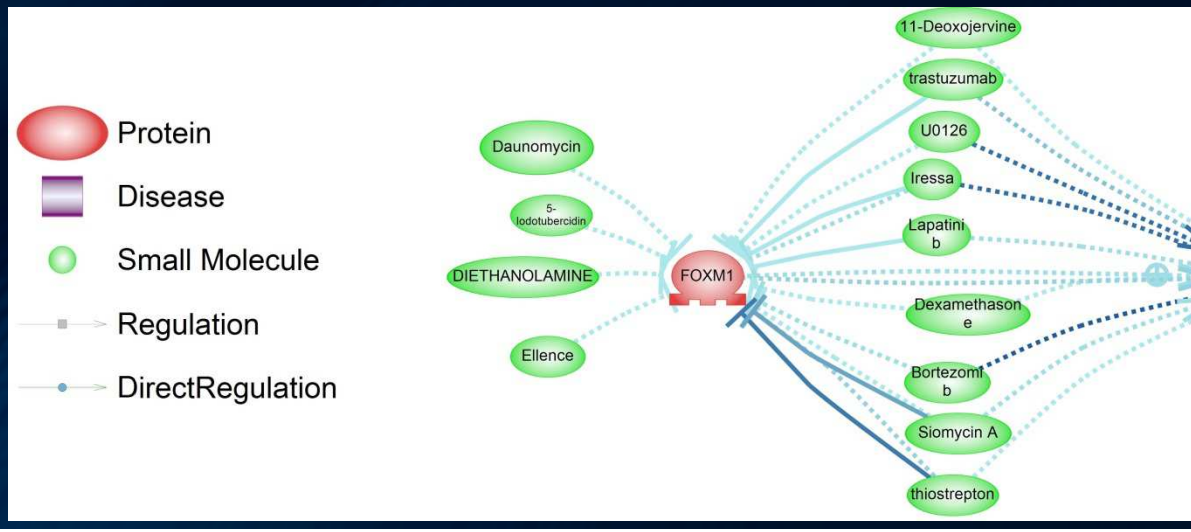
Third patient

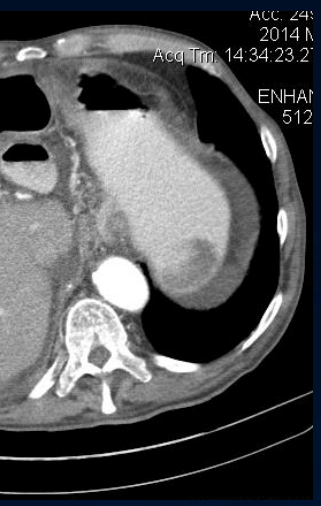
nor signaling pathway



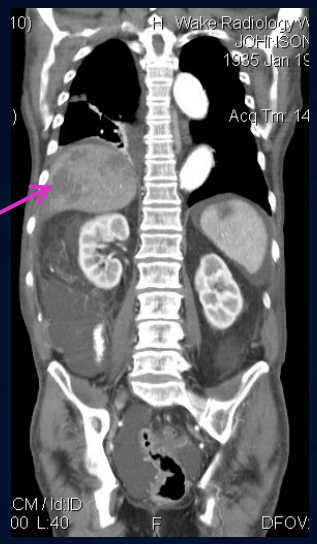






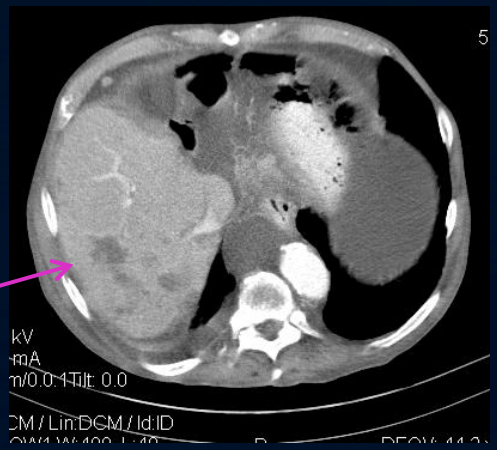


narch-2014
erse CT/Scan

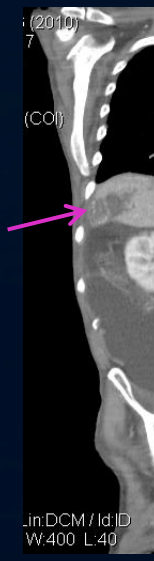


10-march-2014
Coronal CT/Scan

After adding dexamethasone to his regimen, scan shows decreasing number of mets

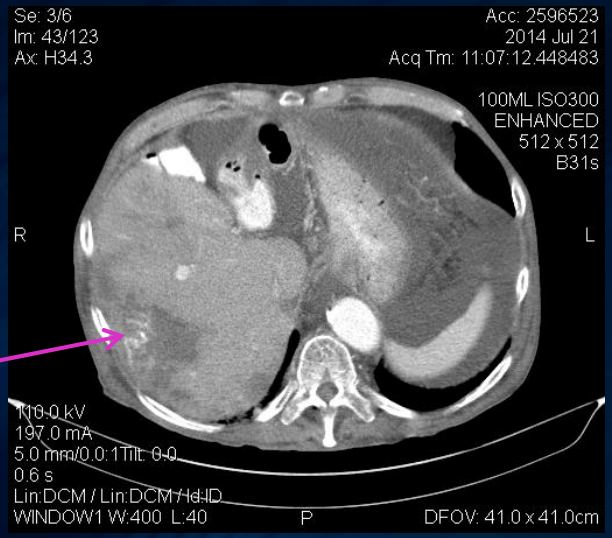


19-may-2014
Transverse CT/Scan

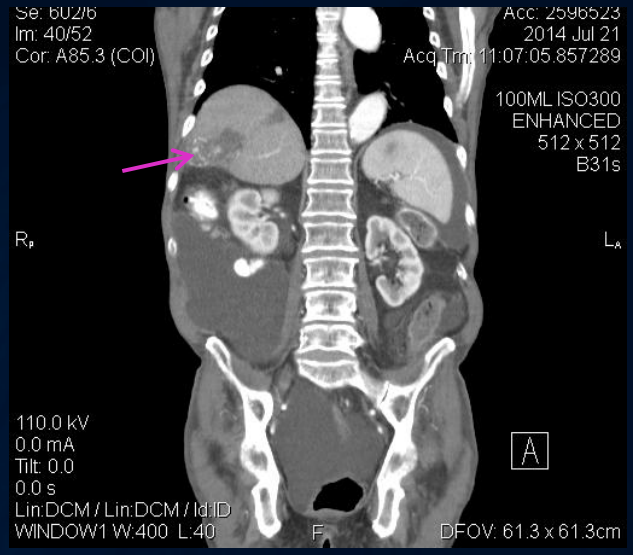


19-m
Coron

r 2 months off chemotherapy shows no increase in number ets but some increase in the size of existing mets



Transverse 21-july-2014 Coronal CT/Scan



Coronal 21-july-2014 CT/Scan

Third patient summary

Patient seen 2 weeks ago is able to walk with a walker, eat meals and enjoy time with family

Patient is still alive with better quality of life at the present time while on a treatment break. He may elect to restart therapy with Ramucirumab

Stage Distribution and Five-year Relative Survival by Stage at Diagnosis for 2003-2009, All Races,		
Stage at Diagnosis	Stage Distribution (%)	Five-year Relative Survival (%)
Localized (confined to primary site) – stage I and II	40	90.3
Regional (spread to regional lymph nodes) – stage III	36	70.4
Distant (cancer has metastasized) – stage IV and recurrent cancer	20	12.5
Unknown (unstaged)	5	33.6

From: http://seer.cancer.gov/statfacts/html/colorect.html#survival

Learning lesson and how a small independent oncology practice will survive in the next 10 years and more

ry patient with metastatic cancer should have gene expression profiling performed on their tu
s to allow the science to dictate which proliferation pathways to block with molecularly target
gs to force apoptosis of their cancer cells, to look at the blueprint of the tumor cells before se
tment – as opposed to using standard of care cytotoxic drugs based on treating a heterogened
ulation of patients with the same cancer without having looked at the tumor blueprint, relying
sician preferences created by guideline committees ("blindfold and pin the tail on the donkey"

atients with metastasis or who exhausted all the treatment options, gene expression profiling
nges the prognosis and management for the majority of patients.

ients for whom the GEP test was performed had longer median survival than that historically
orted for patients with the same diagnosis and stage of disease.

' is projected not only to increase overall survival, but also decrease toxicity of treatment, an
rove quality of life.