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Gene expression profiling for targeted cancer treatment

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Current landscape of cancer care

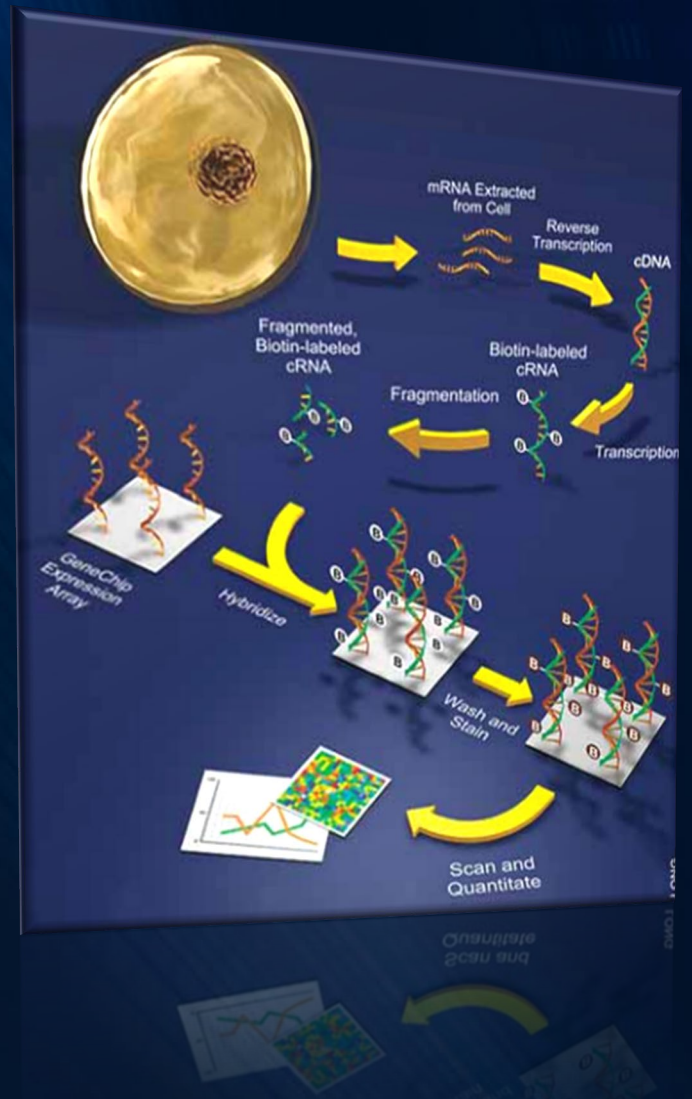
1. In the past 7 years, nearly 900 independent oncology practices in the United States have closed, been acquired by a hospital or merged with another entity, according to the Community Oncology Alliance. Several hundred more are struggling financially.

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.

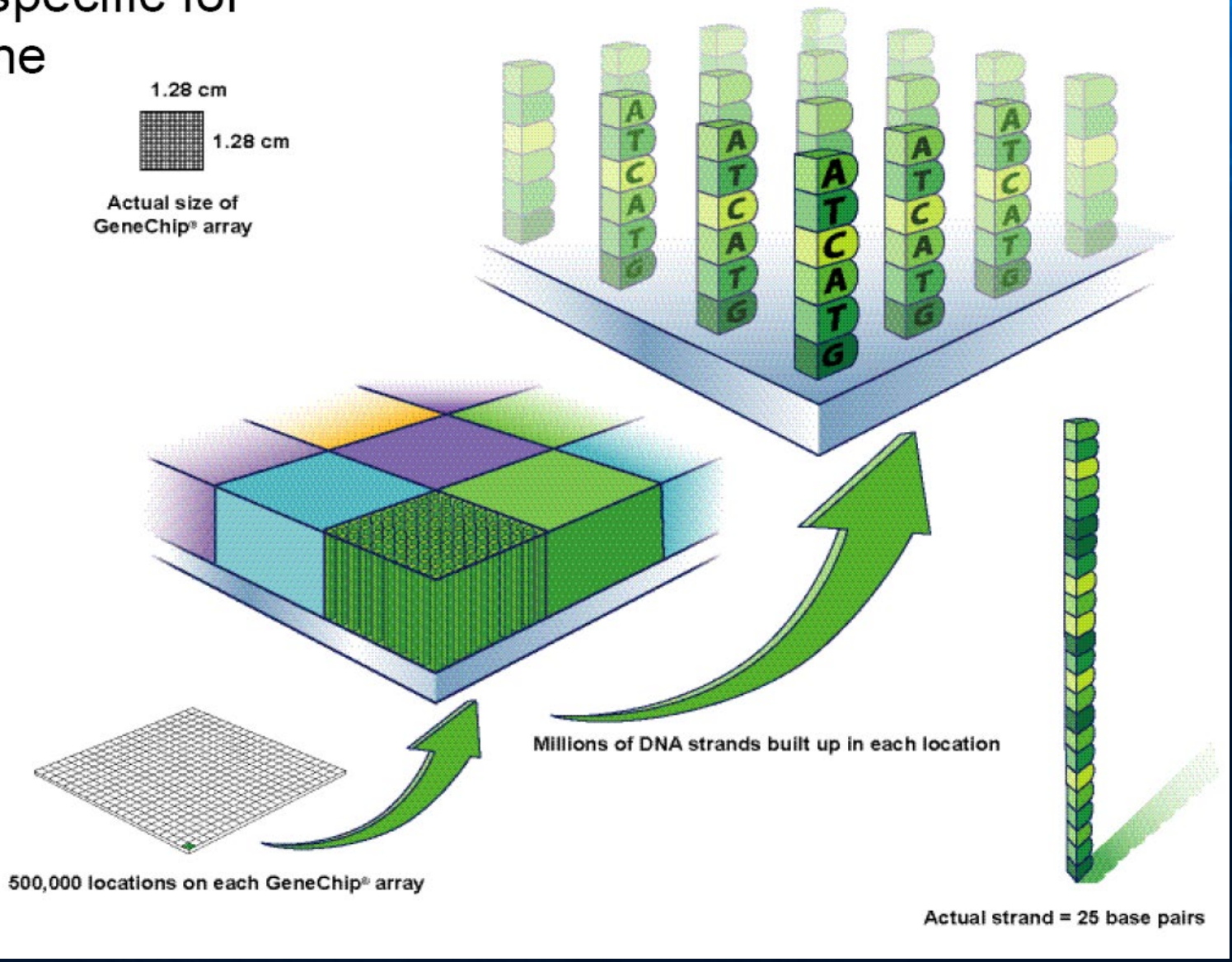
HemOnc today, October 10, 2014

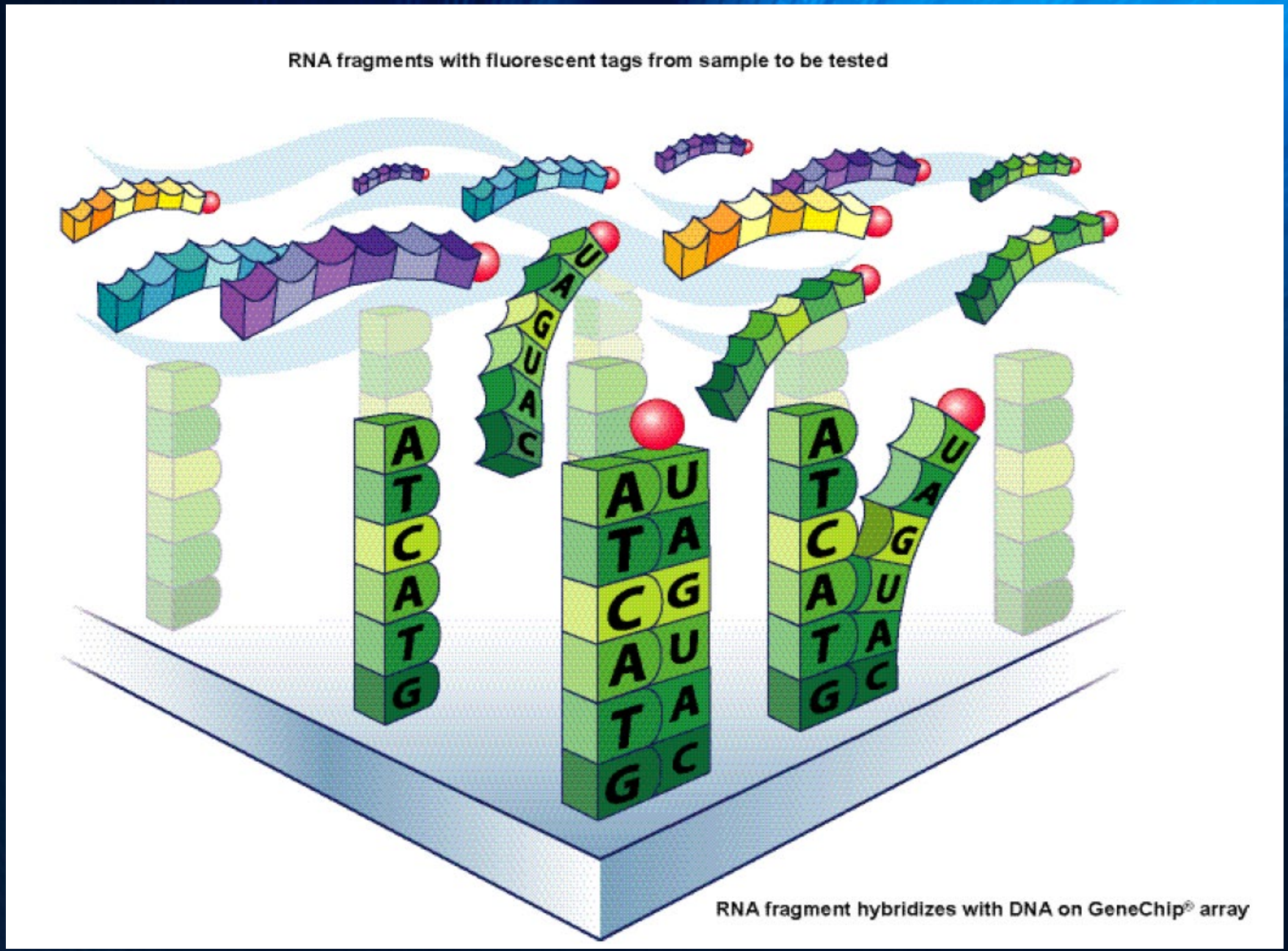
From the research bench to the physician's tool for diagnosis and treatment : Gene expression profiling –> experiment design

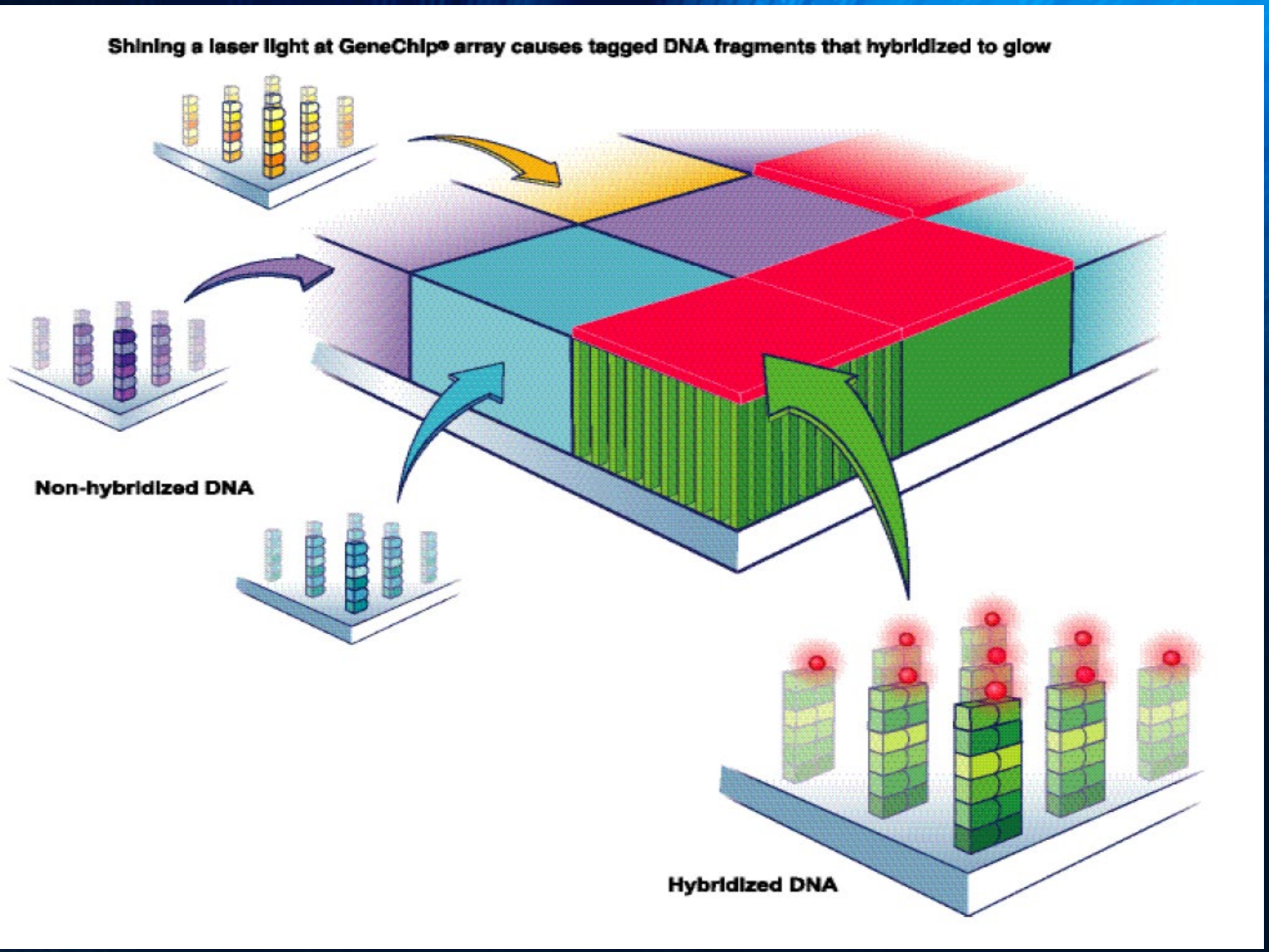


1. core biopsies from tumor and normal tissue preserved in RNA-later
2. RNA extraction and QC - clean, intact RNA will ensure the generation of high quality microarray data
3. synthesis of double stranded cDNA from the RNA sample using reverse transcriptase and an oligo-dT primer
4. in vitro transcription (IVT) reaction that produces amplified amounts of biotin-labeled antisense mRNA (cRNA)
5. cRNA fragmentation using heat and Mg+2 (this fragmentation reduces the cRNA to 25-200 bp fragments)
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 binds to biotin
8. series of washes and stains binds the biotin and provides an amplified floor that emits light when the chip is then scanned and the images processed using Affymetrix software, GeneChip Operating Software (GCOS)
9. MAS file types are generated: Experiment File *.EXP, Image Data File *.DAT, Cell Intensity File *.CEL, Probe Array Results File *.CHP, Report File *.RPT, and MAGE-ML *.XML
10. Importing CEL file into Elsevier Pathway Studio software.

Probe cells – blocks on chip specific for each gene

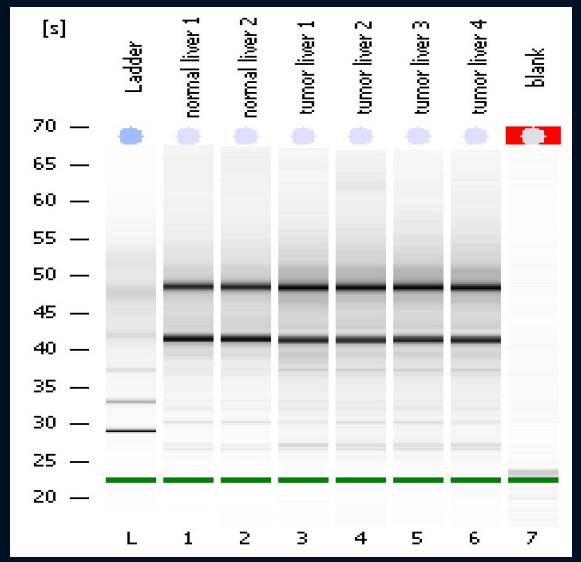


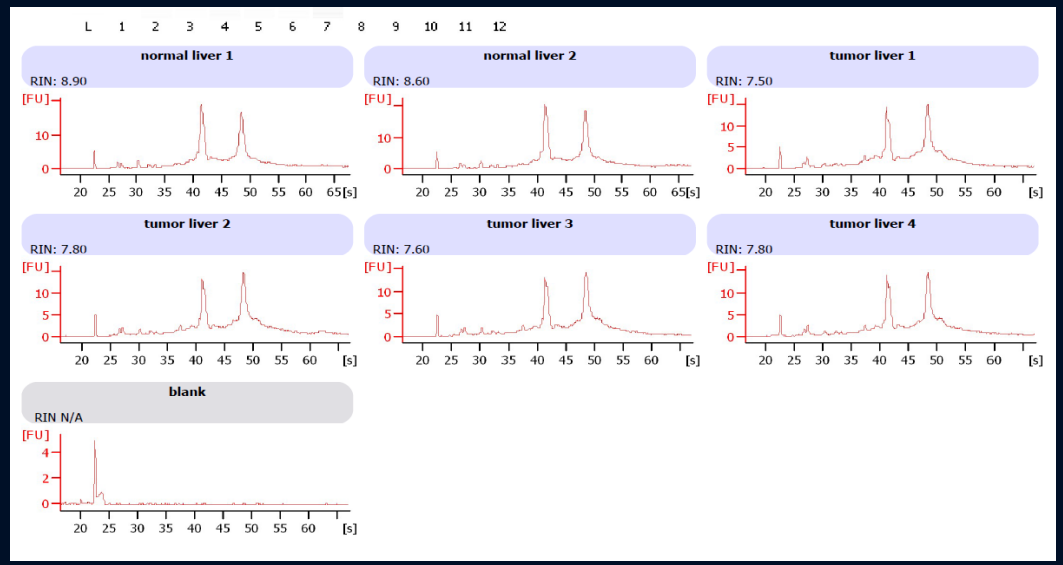

RNA fragments with fluorescent tags from sample to be tested
RNA fragment hybridizes with DNA on GeneChip® array


Shining a laser light at GeneChip® array causes tagged DNA fragments that hybridized to glow
Non-hybridized DNA
Hybridized DNA

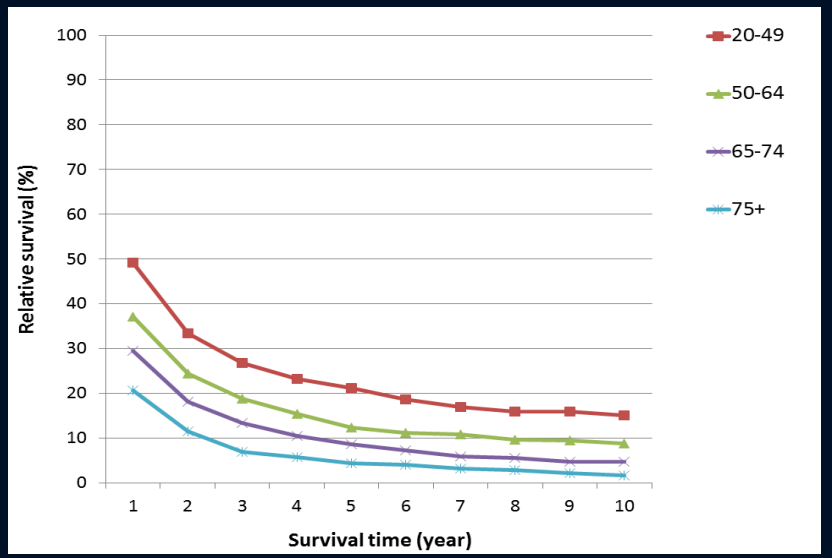
First patient

- 66 year old Caucasian female diagnosed with moderate to poorly differentiated hepatocellular carcinoma with associated necrosis
- Pet/Ct scan shows 9.0x7.2x5.7 cm right hepatic lobe mass
- Resection of hepatocellular carcinoma involving the ascending colon in the right lateral abdominal wall, segments 5 and 6 from the liver and 11 benign lymph nodes
- Core biopsies of liver tumor as well as some of the normal liver parenchymal cells were sent for gene expression profile analysis.



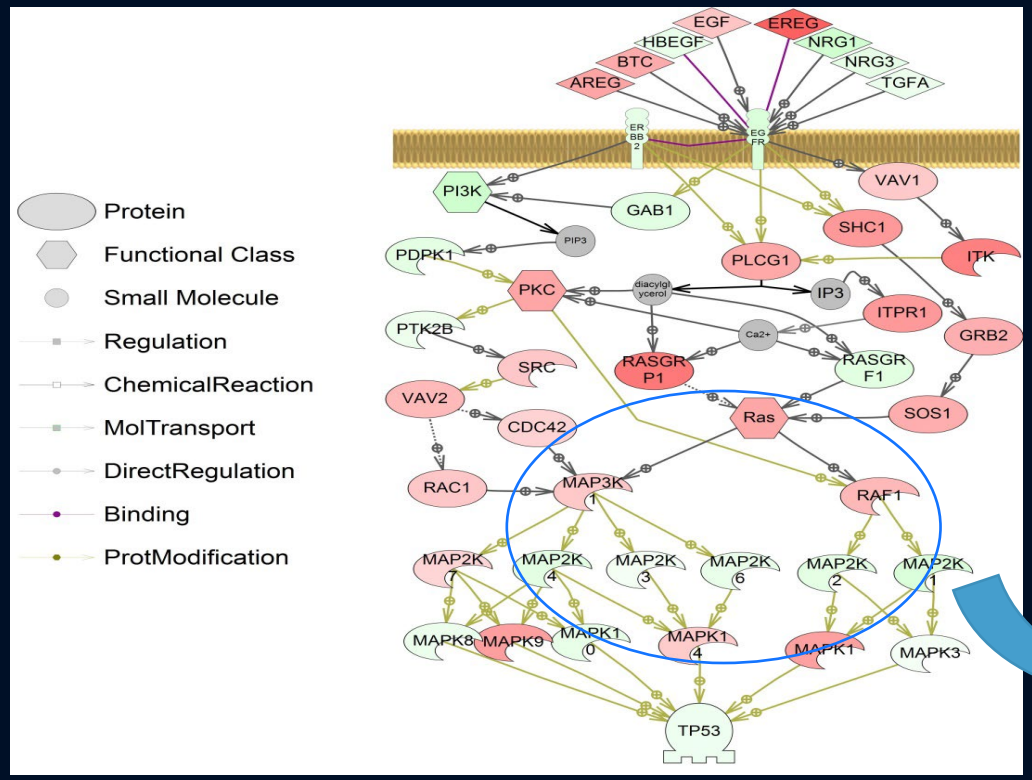


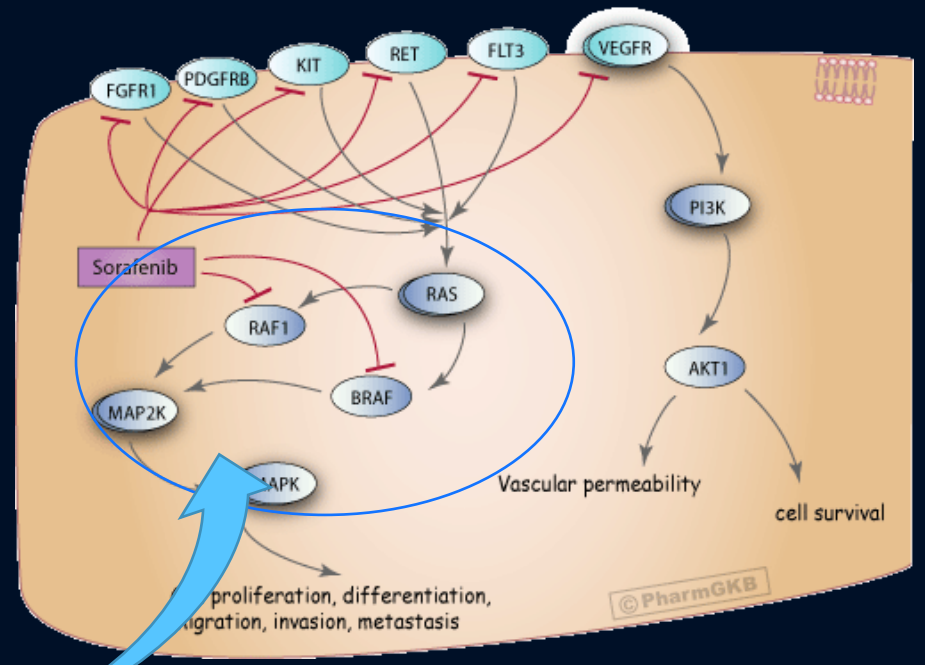
Liver cancer survival in women by survival time and age

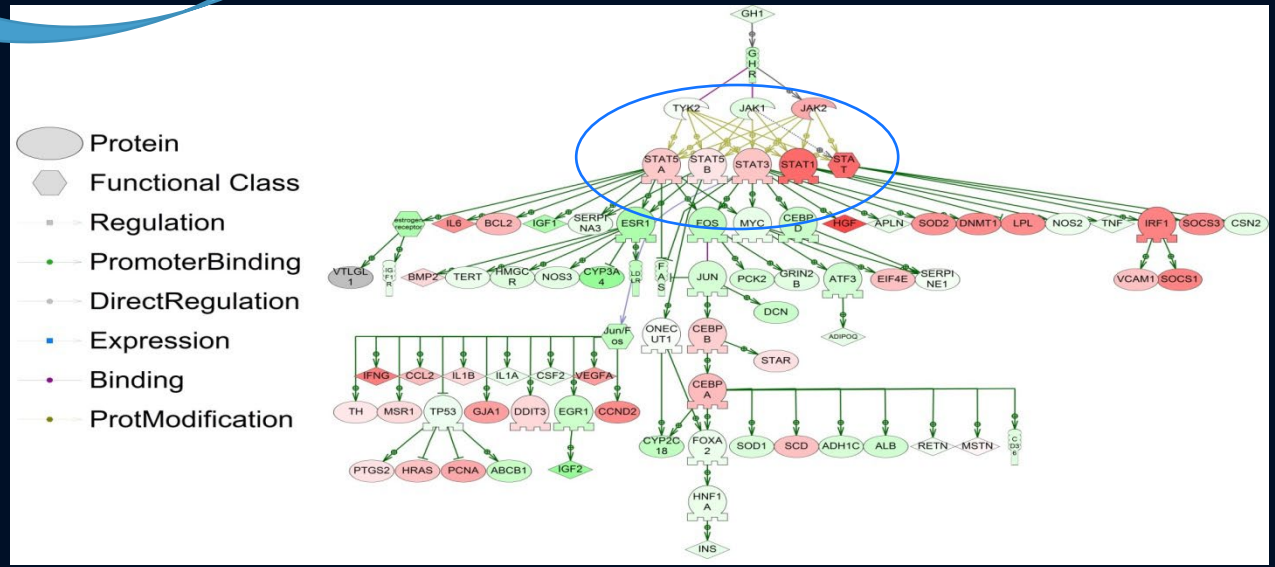


The figure shows survival rates for liver cancer by survival time and age for the US male and female between 1988 and 2007.

Dominant signal transduction pathways through which the hepatocellular carcinoma proliferate is through the Raf- Ras-MEK-Map kinase pathway.



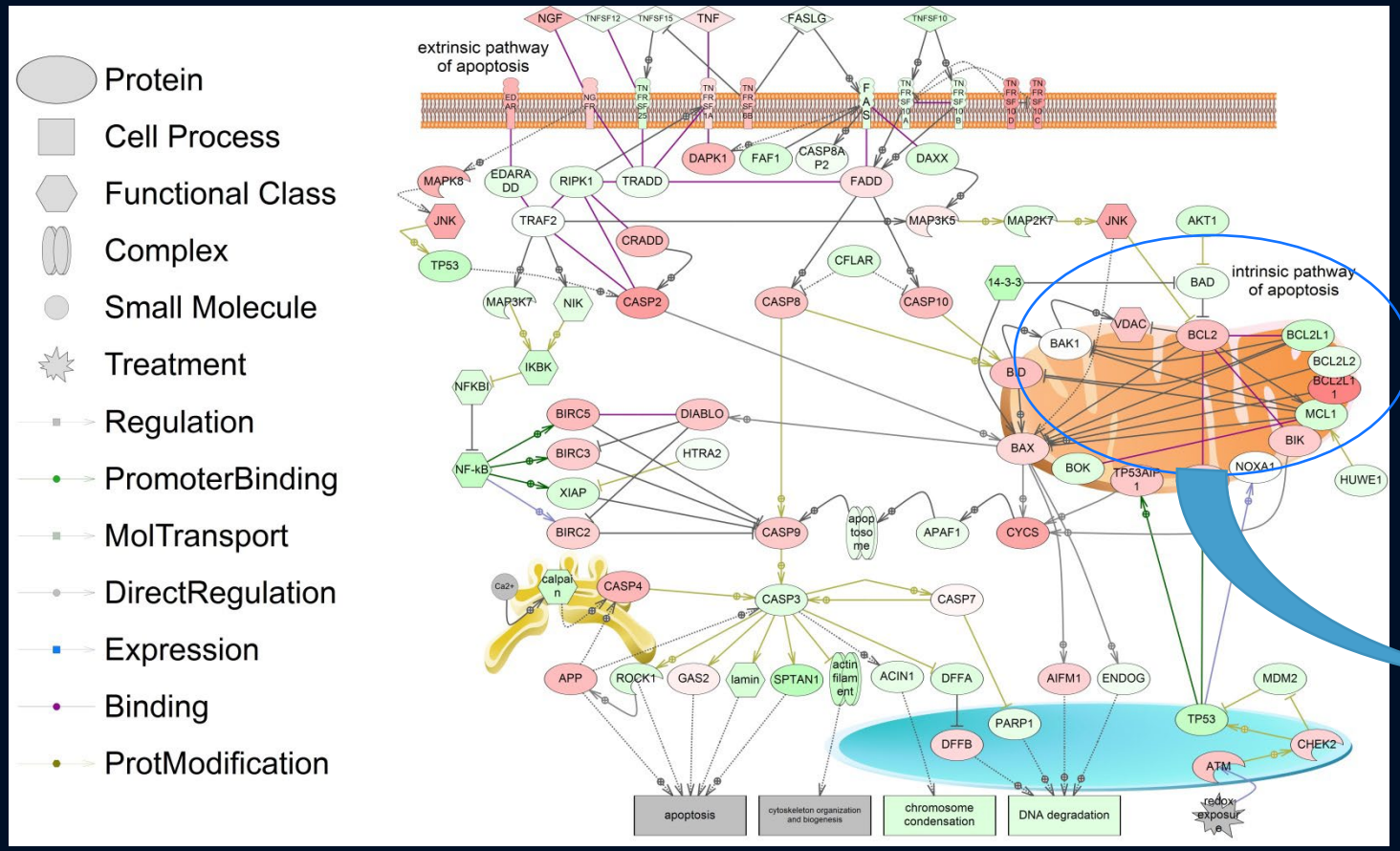


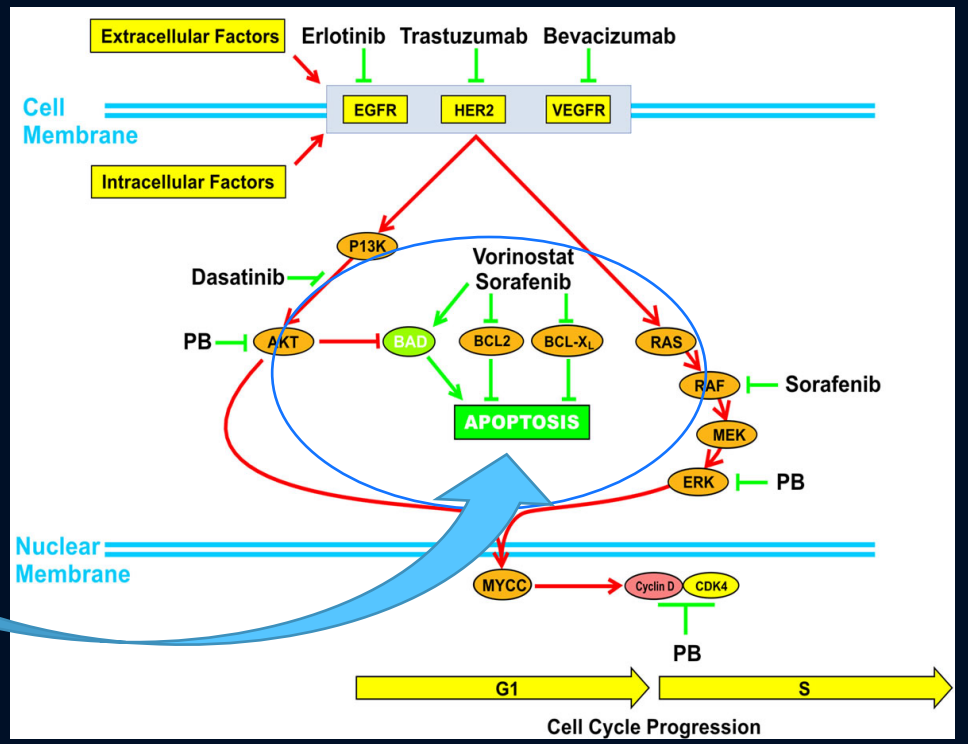


Other pathways within her hepatocellular carcinoma

- ❑ JAK-STAT
- ❑ SMAD – RUNX2
- ❑ AKT1-FOXO1-IGFBP1
- ❑ RUNT – VEGFA

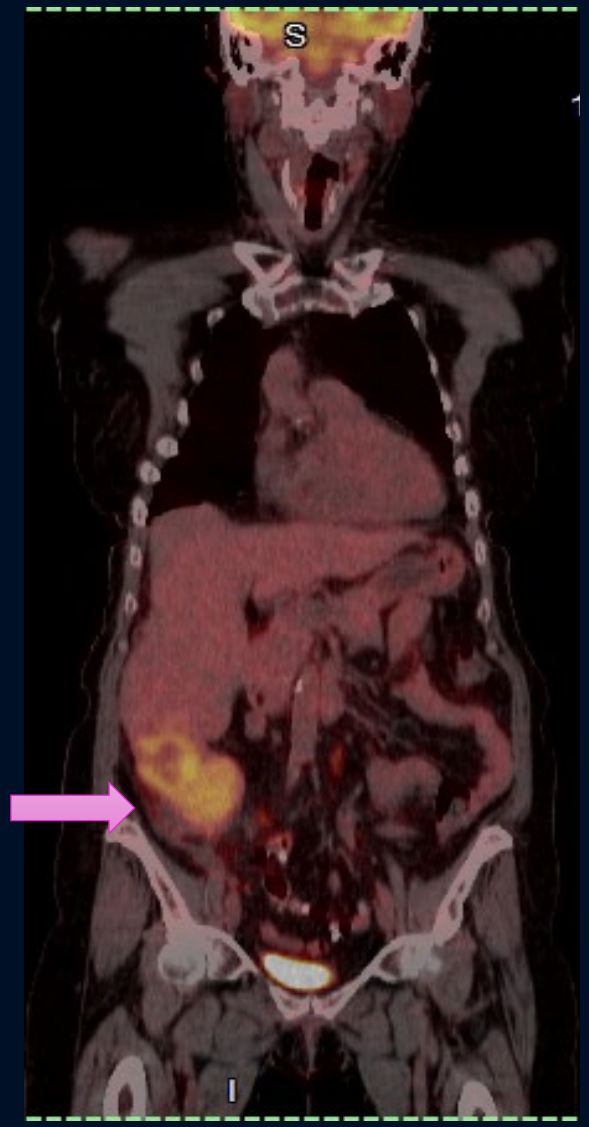
- Patient arranged to receive Sorafenib 400 mg twice a day at least six months
- Resection of residual disease with gene expression profile using mesenteric lymph node material from small bowel
- Patient referred to Virginia, Massey Cancer Center to look at being randomized in the clinical trial (ClinicalTrials.gov Identifier: NCT01075113) looking at combinations of molecular targeted therapies inducing apoptosis (Vorinostat) in addition to the dominant pathway, which were blocked by Sorafenib: EGFR–Ras–Raf– MEK–Map kinase–ERK pathway.



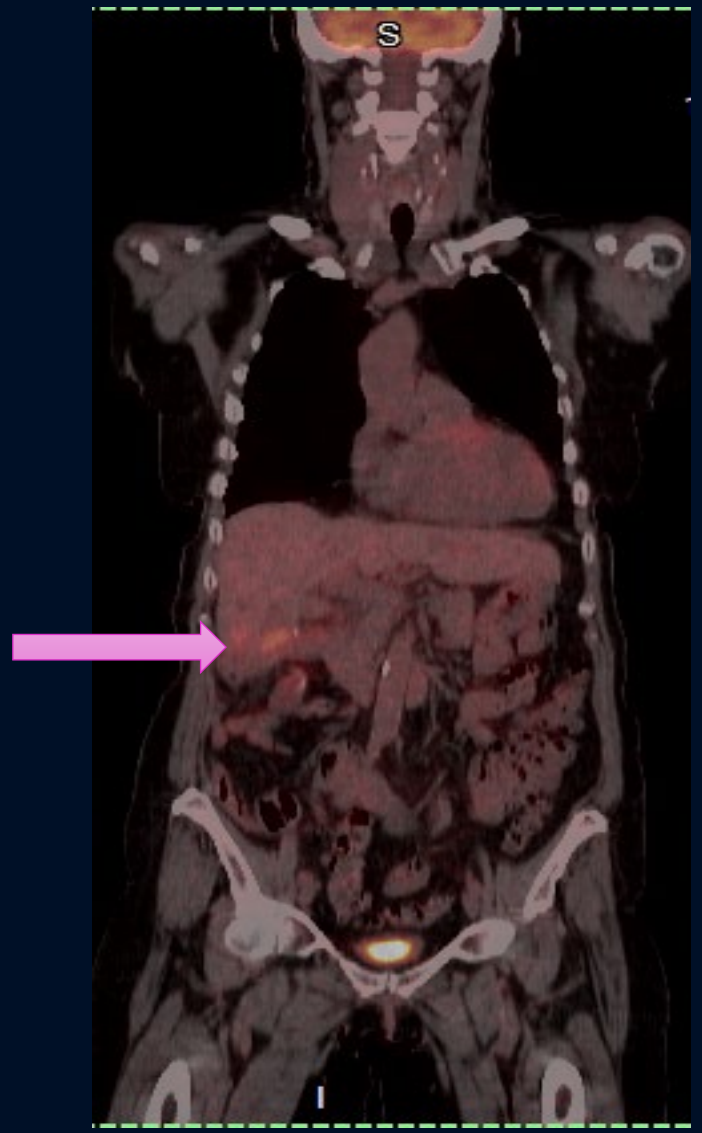


Purpose: This phase I trial is studying the side effects and best dose of vorinostat when given together with sorafenib tosylate in treating patients with advanced liver cancer. Sorafenib tosylate and vorinostat may stop the growth of tumor cells by blocking some of the enzymes needed for cell growth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

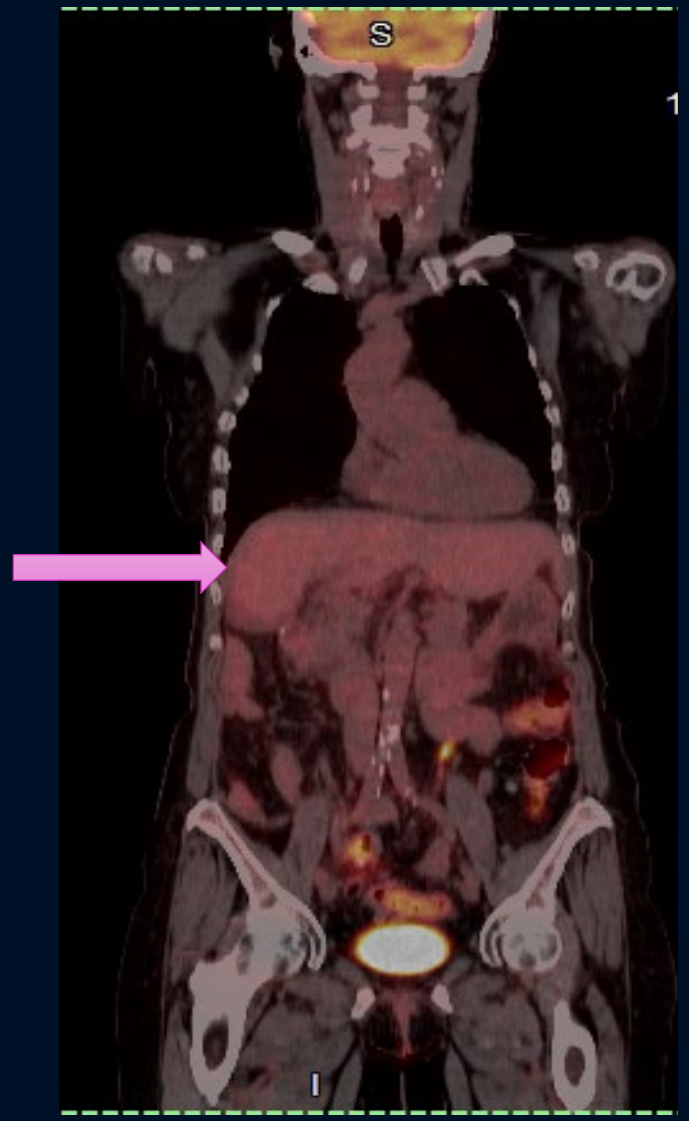
Before and after treatment



Before treatment
PET/Scan-July, 2013

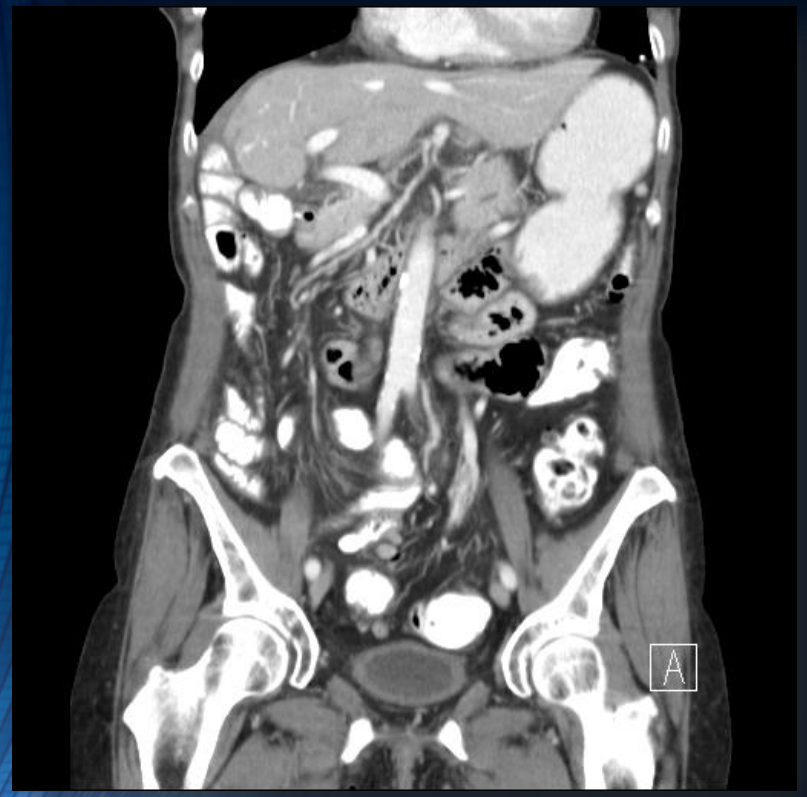


After resection and **Sorafenib** 400 mg twice a day (six months)-PET/Scan



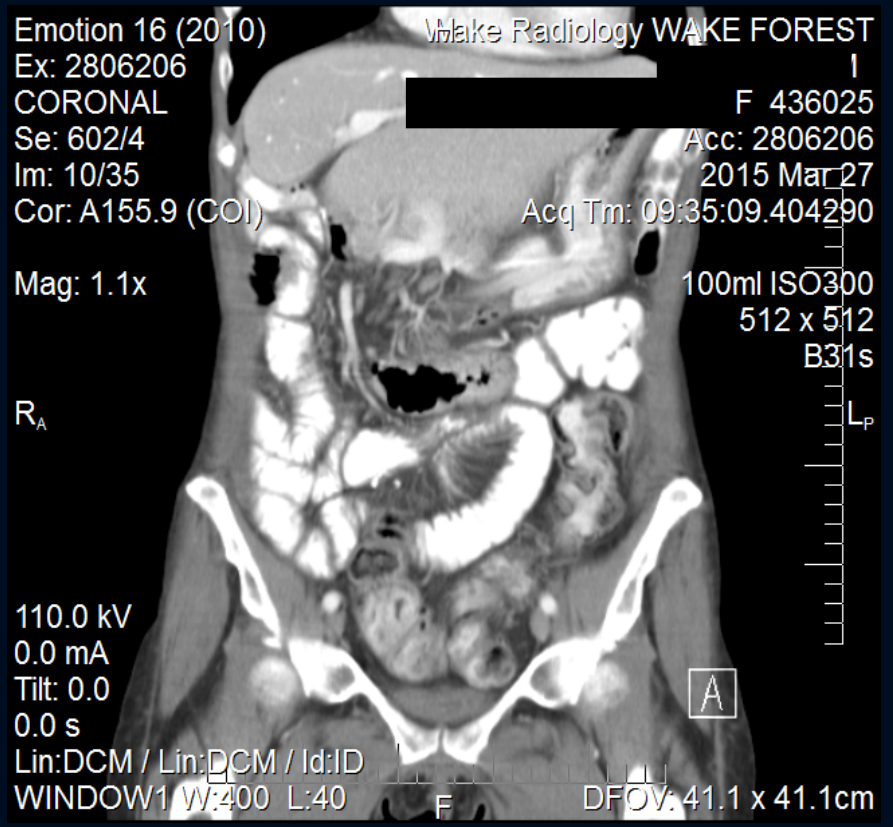
After **NCT01075113 clinical trial Sorafenib plus Vorinostat**
Massey Cancer Center, Virginia, June 2014-PET/Scan

First patient summary



CT/Scan from 10-October-2014

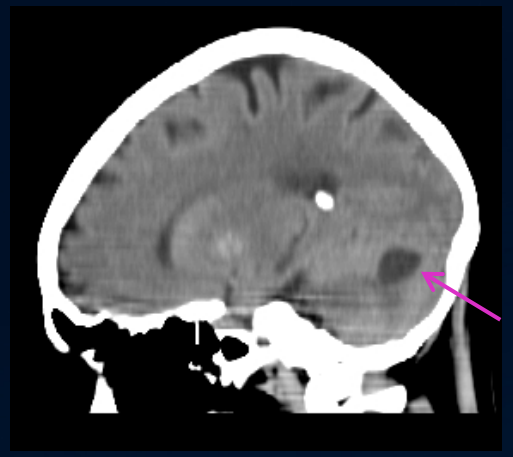
- ✓ Continue the clinical trial
- ✓ Patient has outlived Overall Survival estimates based on standard of care treatment and continues to have normal quality of life with intermittent grade I hand–foot syndrome from her Sorafinib + Voronistat treatments on Clinical trial and to this date in time appears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



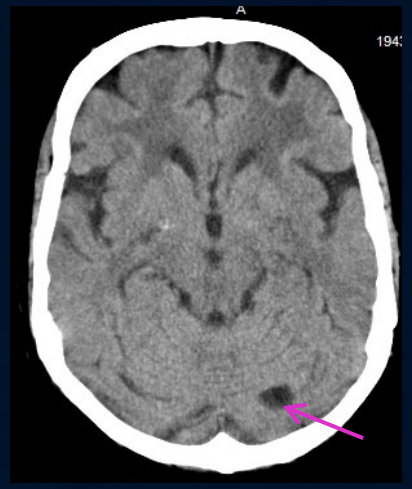
CT/Scan from 27-March-2015

Second patient

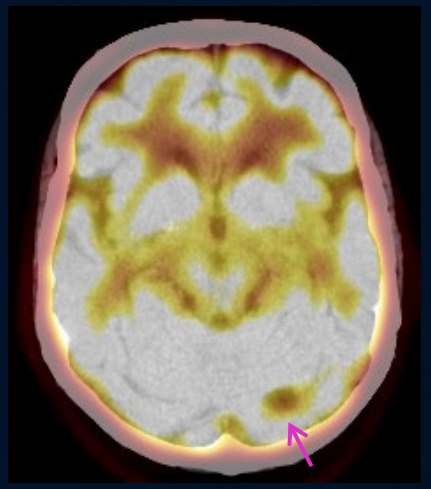
- 66 year old Caucasian female diagnosed in 2011 in Florida with stage I breast cancer, miss-labeled as ER+ /PR+ , and treated with Docetaxel/Cyclophosphamine (4 cycles) followed by hormonal therapy
- Cancer recurrence in 2013 and diagnosed with stage IV breast cancer with mets in the right lung and her brain (diagnose made in our practice for the first time after moving to North Carolina)
- Re-diagnosed (initial tumor block from Florida) as ER-/PR- and treated with radiation for the brain met and 2 cycles of Adriamycin/Cyclophosphamide (dose dense standard of care therapy, based on ASCO and NCCN guidelines) for the breast cancer lung mets
- Brain met responded to the radiation treatment but the lung met did not respond to AC chemotherapy and core biopsies were performed from the lung met
- Treatment was switched to Gemcitabine (standard of care) until the gene expression profiling data was processed



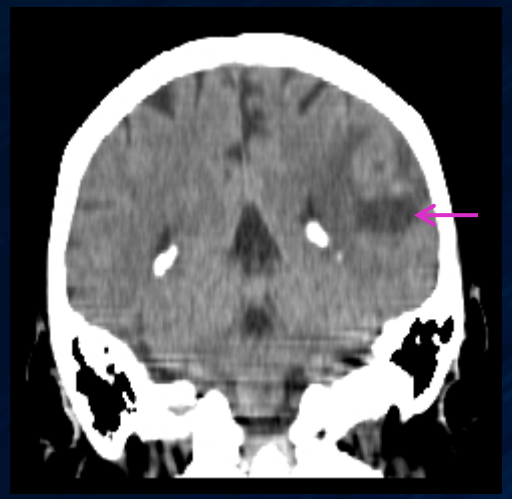
12-sept-2013: left **cerebellum** met CT/Scan



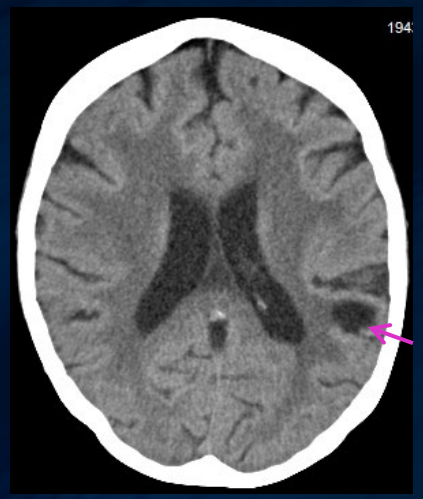
8-july-2014: left **cerebellum** no change in size CT/Scan



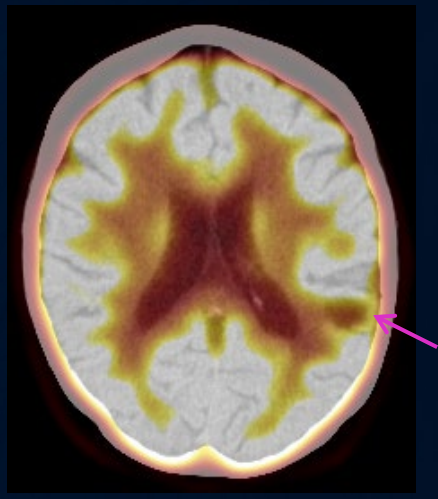
8-july-2014: left **cerebellum** no increased hyper-metabolic activity PET/Scan



12-sept-2013: left **parietal** met CT/Scan



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



8-july-2014: **left parietal** no increased hyper-metabolic activity PET/Scan

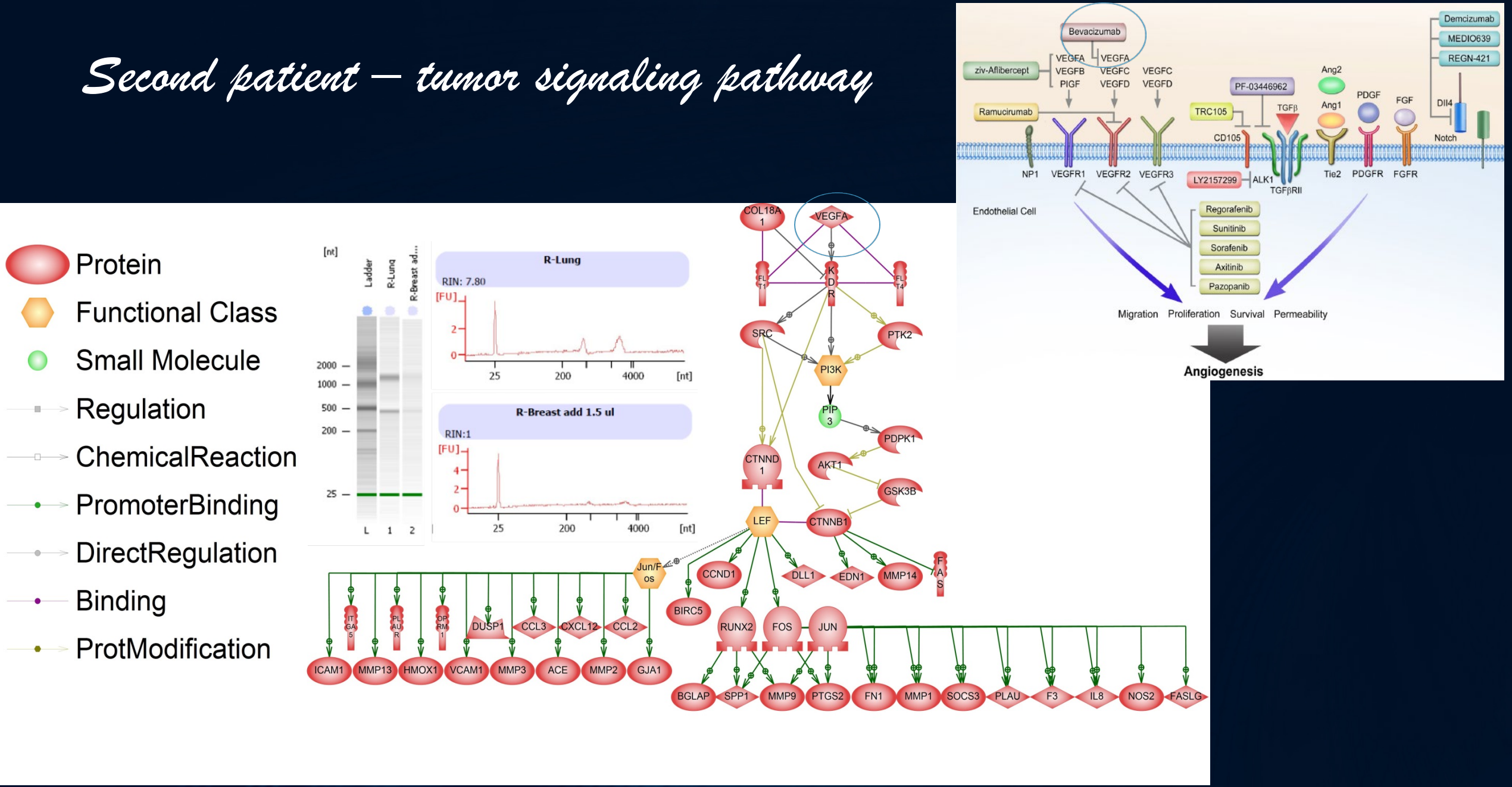
- Gene expression profiling reveals:
  - ER -, PR-, Her2Neu- confirming pathology findings as a triple negative breast cancer metastasis
  - Cell invasion through: connective tissue growth factor (CTGF)->fibronectin 1 (FN1) ->integrin α-5 pathway
  - Cell cycle is activated through transcription factor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

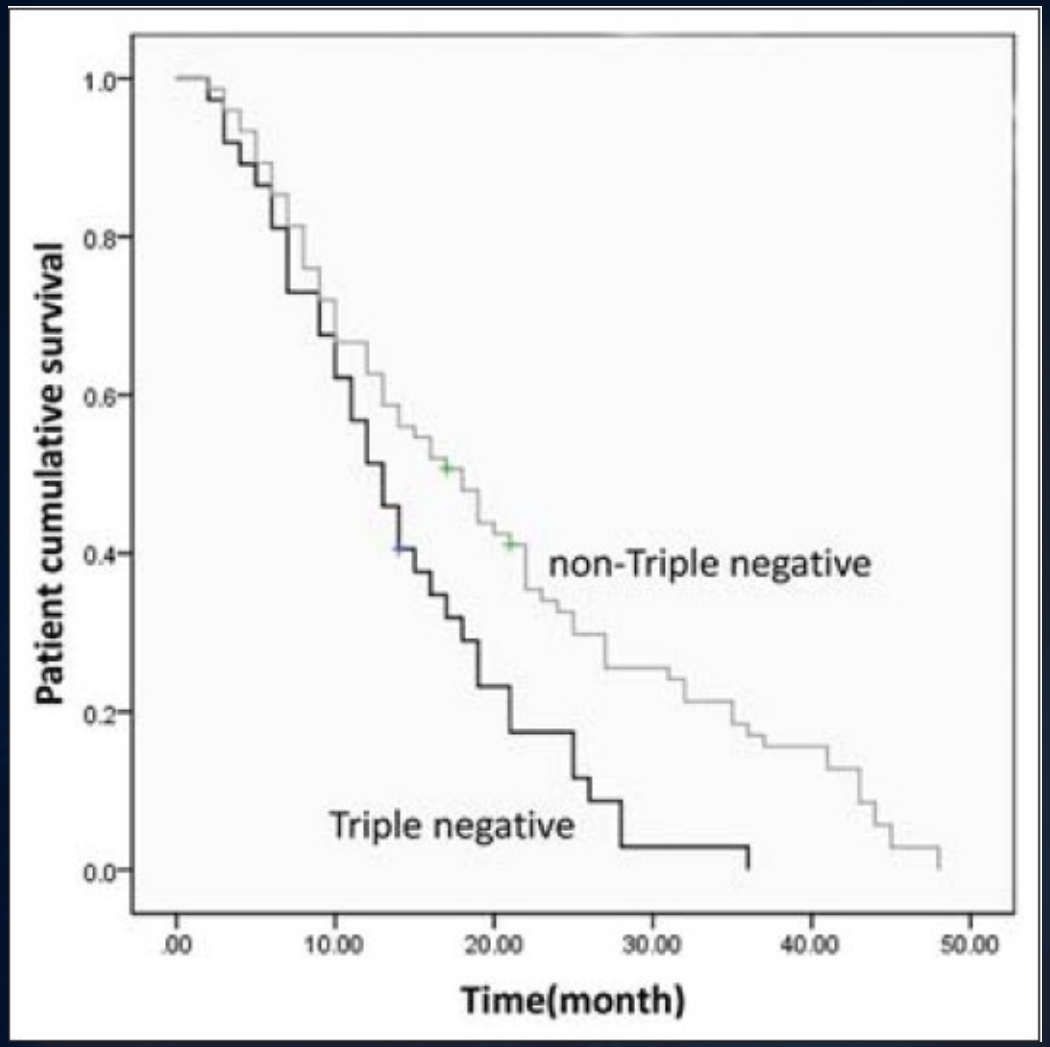
- Based on the tumor pathways , bevacizumab (recombinant human monoclonal antibody that blocks angiogenesis by inhibiting VEGFA) was added to her treatment to decrease blood vessel ingrowth to her tumor from angiogenesis

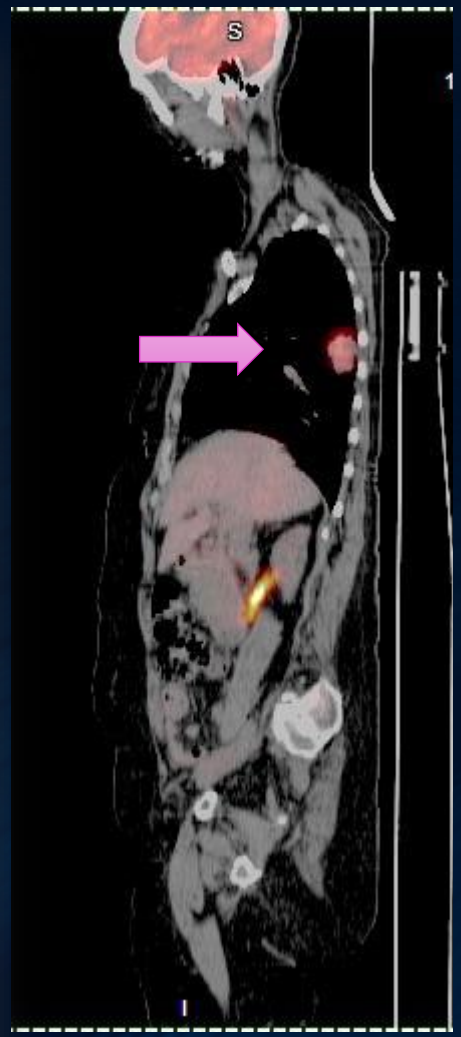
- Repeated scan showed considerable decreasing on the lung met to the size that allowed it to be resected follow up by 2 more doses of bevacizumab plus Gemcitabine

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

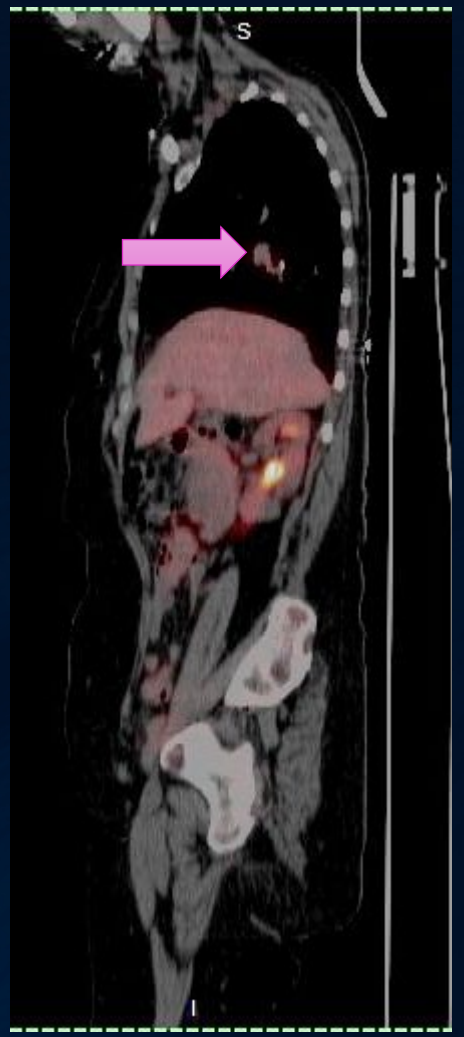
Second patient – tumor signaling pathway



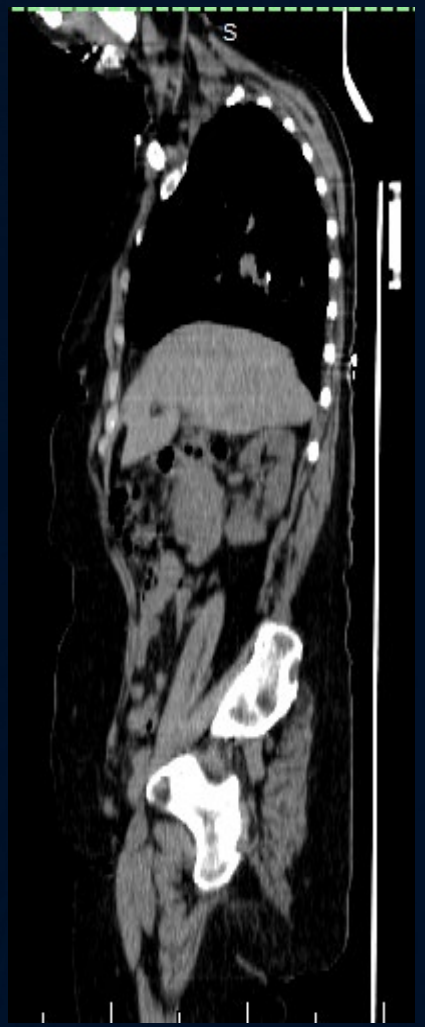




Lung met
before treatment
12 September 2013
PET/Scan



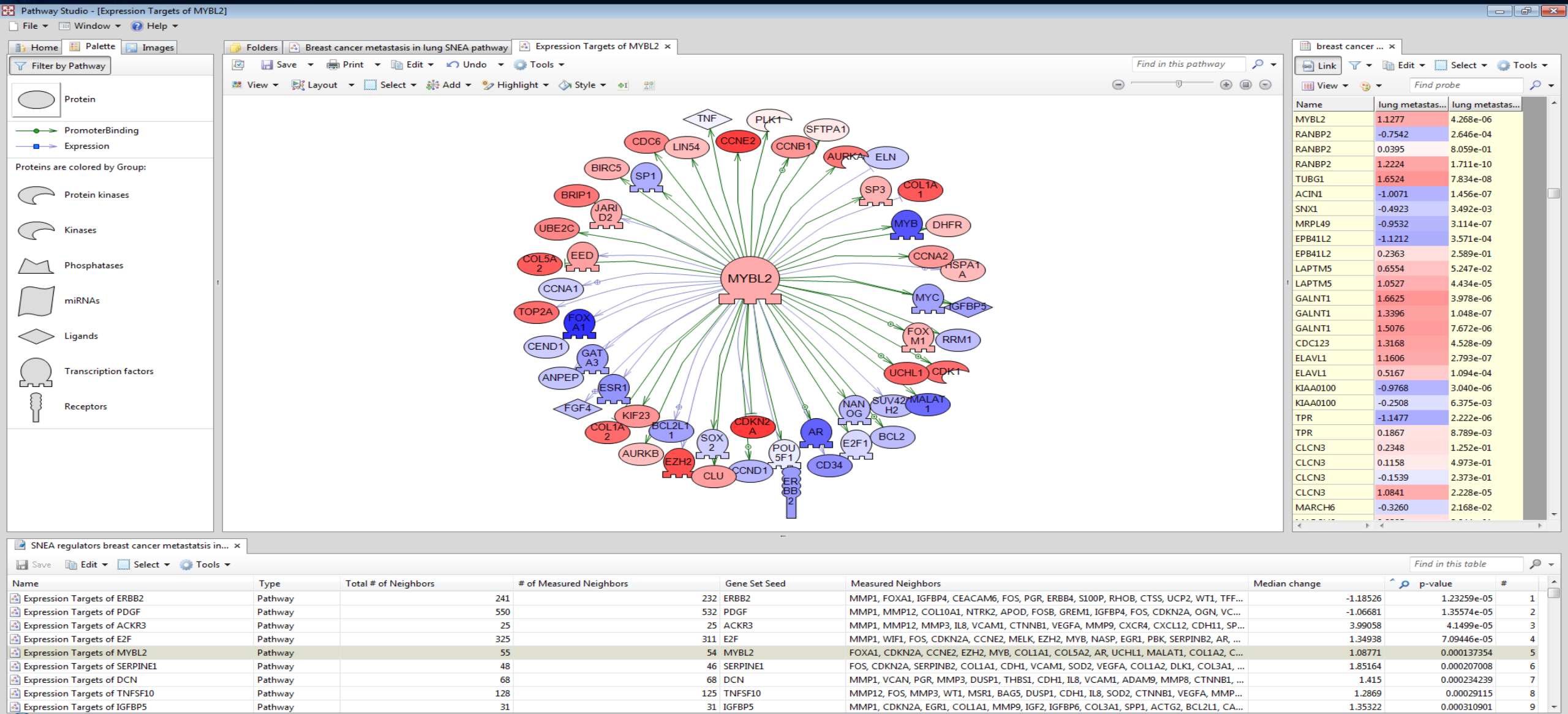
No lung met
after treatment
8 July 2014
PET/Scan



No lung met
after treatment
8 July 2014
CT/Scan

Step1: Import gene expression profile and run sub-network enrichment analysis (SNEA) to find top 100 expression regulators

SNEA identifies regulators that have most differentially expressed targets

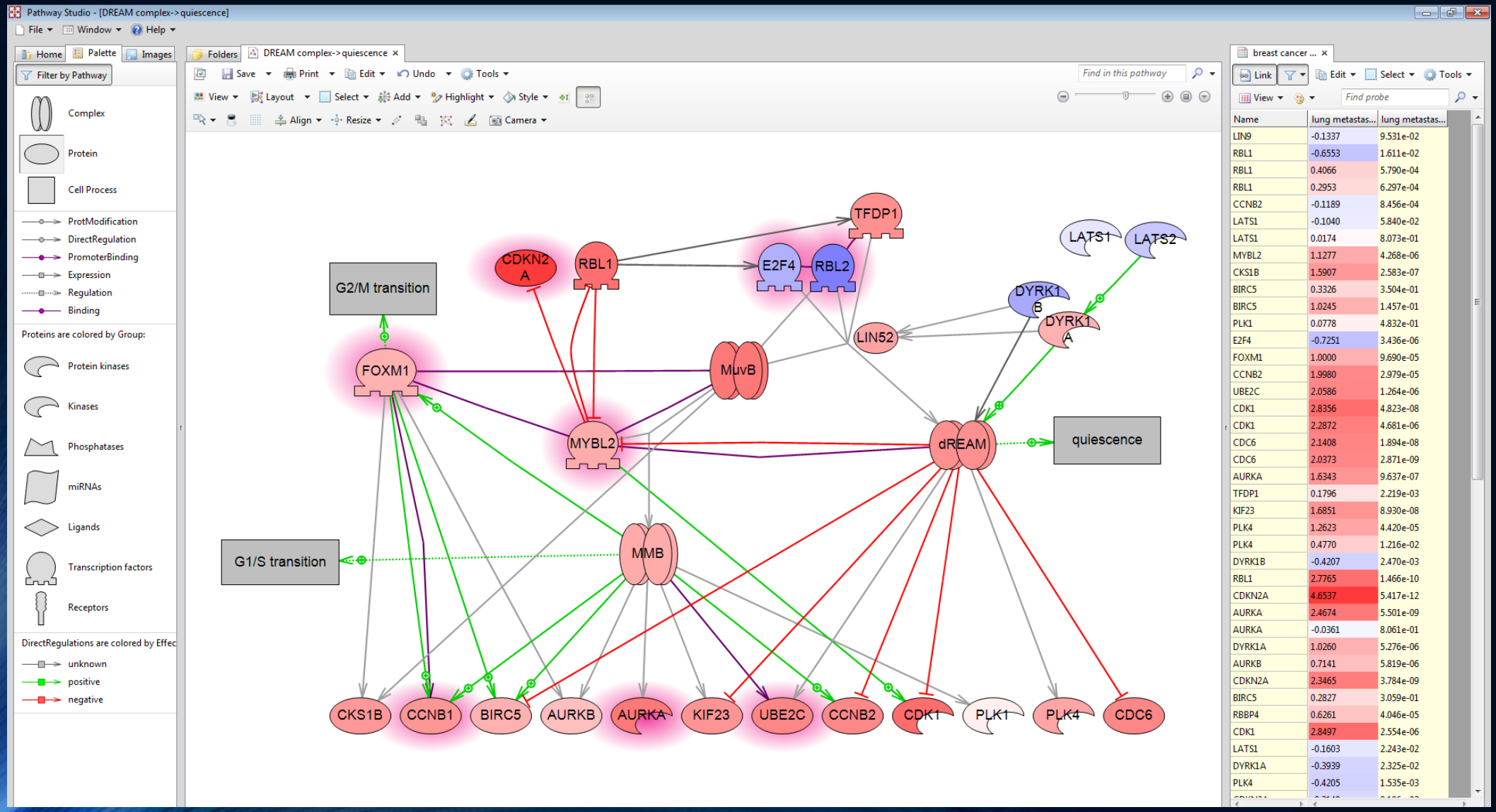


STEP2: Find pathways enriched with expression regulators identified at STEP1

Top 100 expression regulators
Identified at STEP1 arranged by their cellular localization

STEP 3: Identify potential drug targets as major expression regulators belonging to one of the top pathways identified at STEP 2

DREAM complex pathway was identified as top pathway activated in patient with breast cancer metastasis in lung. Highlighted in red – major expression regulators from STEP 1



DETOURS

1. What if there are too many drugs for selected pathway?

   Use additional constrains; select only drug with shown efficacy against targeted type of cancer

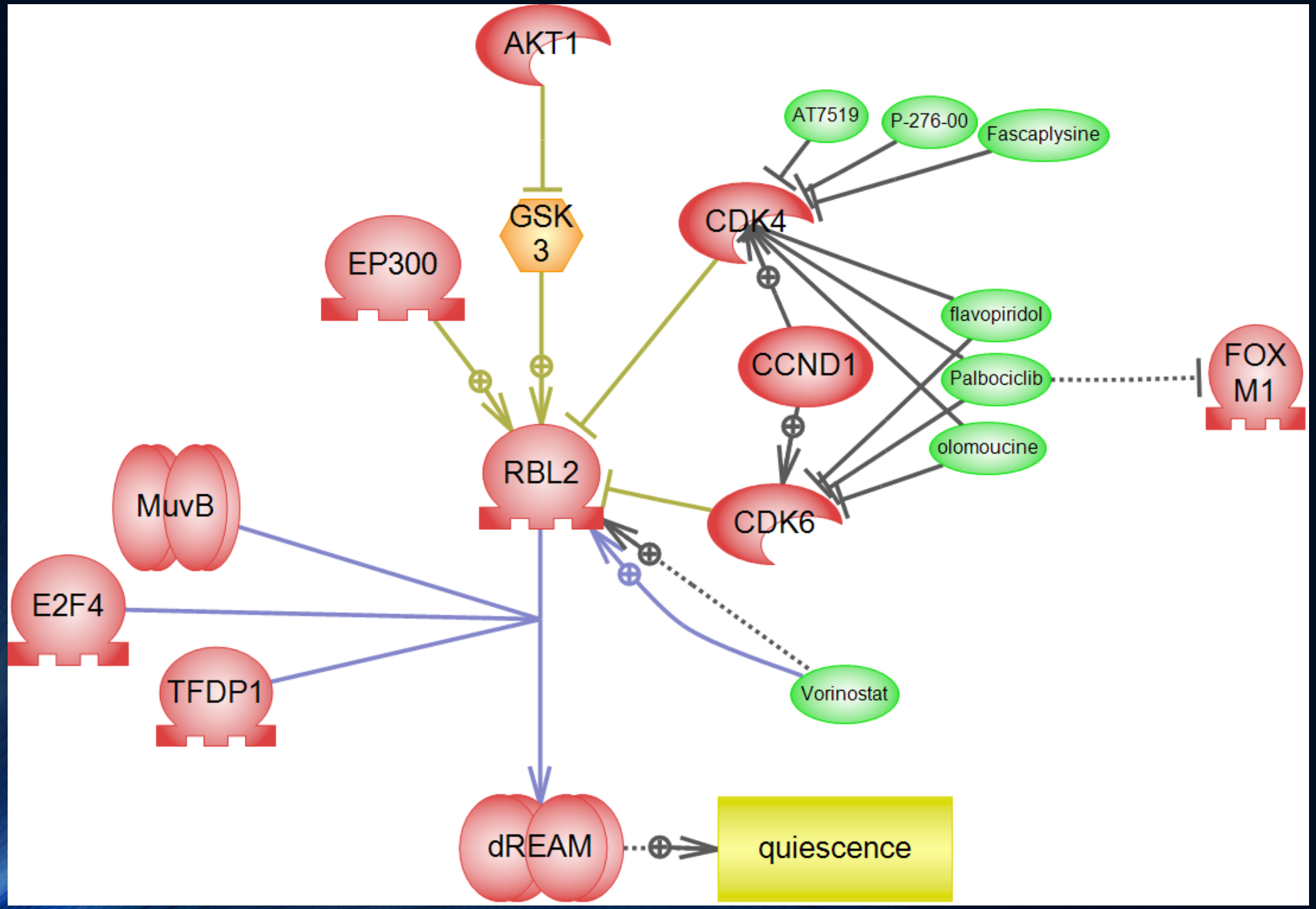
2. What if you cannot find pathways enriched with expression regulators?

   You have to build new pathway from available expression regulators. Building new pathways requires expertise. The Expertise is available at Personalized Hematology/Oncology of Wake Forest to build pathways

3. What if top pathway does not have good drug targets?

   You can look for targets in other top 10 pathways found at STEP 2
   You can perform pathway analysis and find targets upstream of the pathway of interest
   Pathway depicts the molecular mechanism of a biological process. You can find drugs inhibiting the process rather than drugs inhibiting the pathway. There are more data available on how drugs inhibit cell processes than on drug targets

Drugs regulating DREAM complex pathway identified by upstream pathway analysis



Second patient summary

- ✓ Patient developed clinical signs or symptoms of disease progression PET/CT scan 7 November 2014, CT-guided biopsy of new liver metastasis sent for gene expression profiling

- ✓ Patient developed 80% vision loss in her left eye in December 2014, unable to perform MRI of brain after seeing Neuro-Ophthamologist due to pacemaker wires, unable to visualize any new brain mets on CT scanning

- ✓ Tumor characteristics had changed to a new dominant pathway utilizing IP3-Akt pathway
- ✓ Patient switched to Pablociclib 100 mg PO daily for 21 days of a 28 day cycle

- ✓ Patient just prior to initiation of Pablociclib in March 9, 2015, developed intractable nausea & vomiting – CT scan of brain on March 10, 2015 revealed new satellite cerebellar brain mets & two new mets now visibly present on CT scan near optic tract; patient placed on Decadron overnight with resolution of intractable nausea & vomiting

- ✓ Patient sent to Neurosurgeon, who performed a cerebellar metastatectomy and sent to UNC for cyberknife of two metastatic lesions near her optic tract

- ✓ *Patient has outlived Overall Survival estimates based on standard of care treatments and continues to have near normal quality of life while undergoing treatment on Pablociclib*

Third patient

- 74 year 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

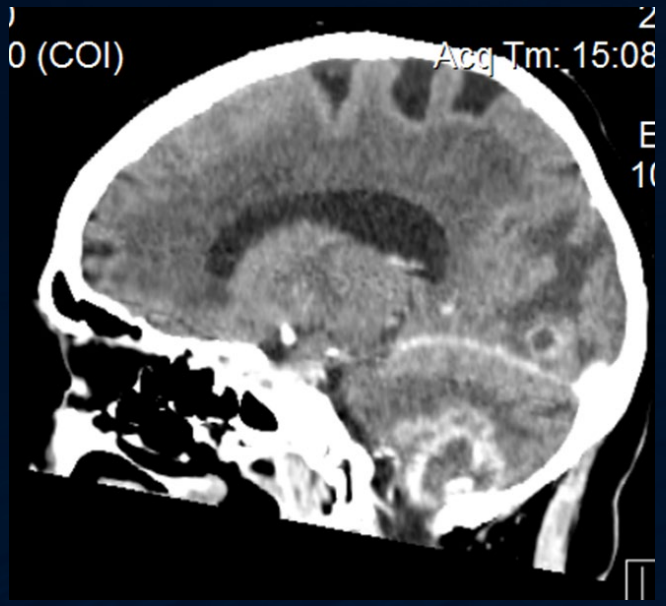
- Cancer recurrence in 2013 with multiple mets in the liver and lung; core biopsies were performed from the liver met for gene expression profiling

- In January 2014 started standard of care modified FOLFOX6 regimen every 2 weeks with 5-FU CADD pump
  - FOLFOX6 = 5-FU + Oxaliplatin + Leucovorin

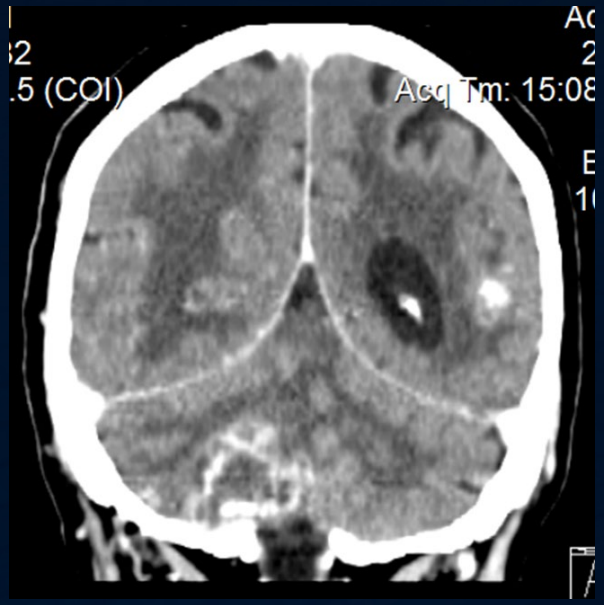
- Gene expression profiling reveals:
  - tumor grows due to mitotic activation
  - FoxMP1 activation in the tumor ->FoxMP1 confers resistance to many drugs including chemo drugs
  - drug metabolization pathways are active -> bad outcome because tumor adapts to liver and liver's function is drug metabolism.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

- Based on the tumor pathways
  - Dexamethazone (blocks FoxMP1) was added to his original treatment (FOLFOX6) but without oxaliplatin to decrease 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 VEGF 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 with benefit in overall survival

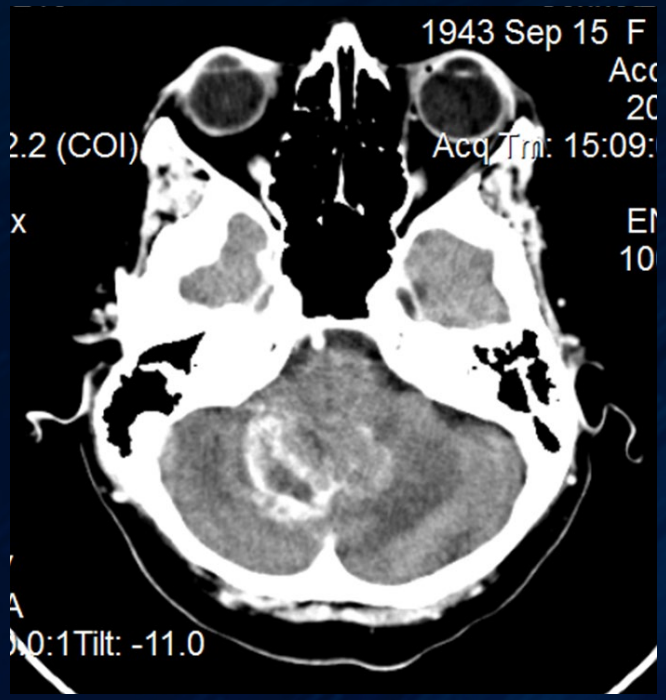
- June and July 2013 the patient was on hold from the toxicity of chemo treatment due to: typhilitis, pneumotosis of the colon (air in the colonic wall) and pulmonary embolism; started back on treatment in August 2014 but after 3 cycles decided to not have any more chemotherapy at that time.



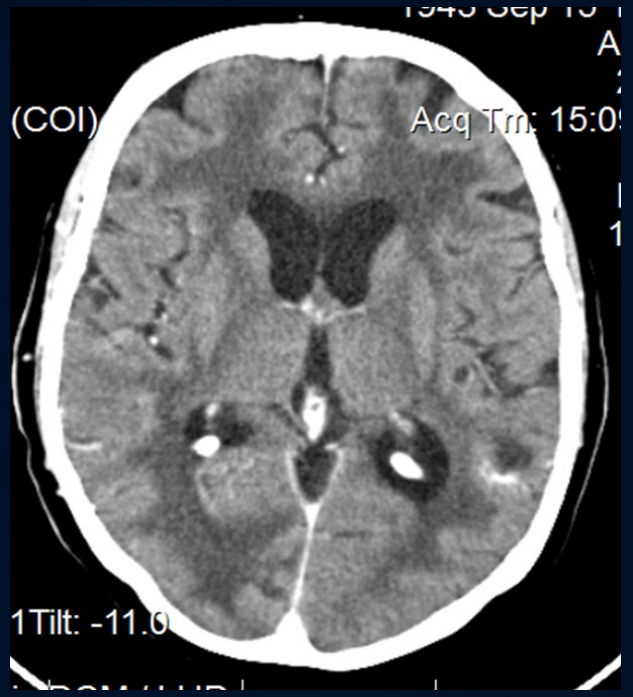
10-march-2015:
left **cerebellum** met
CT/Scan



10-march-2015:
left **cerebellum** met
CT/Scan

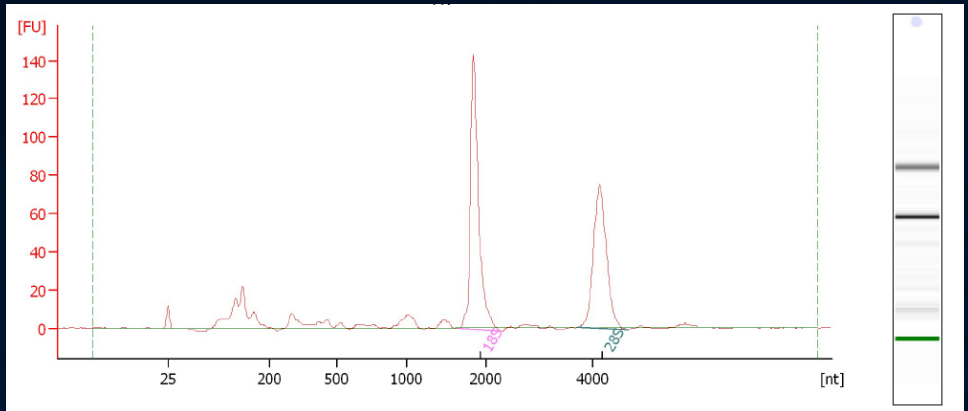


10-march-2015:
left **parietal** met
CT/Scan



10-march-2015:
left **parietal** met
CT/Scan

Third patient tumor signaling pathway



Overall Results for sample 11 : MV

RNA Area:	486.8	RNA Integrity Number (RIN):	6.9 (B.02.08)
RNA Concentration:	490 pg/µl	Result Flagging Color:	
rRNA Ratio [28s / 18s]:	0.7	Result Flagging Label:	RIN: 6.90

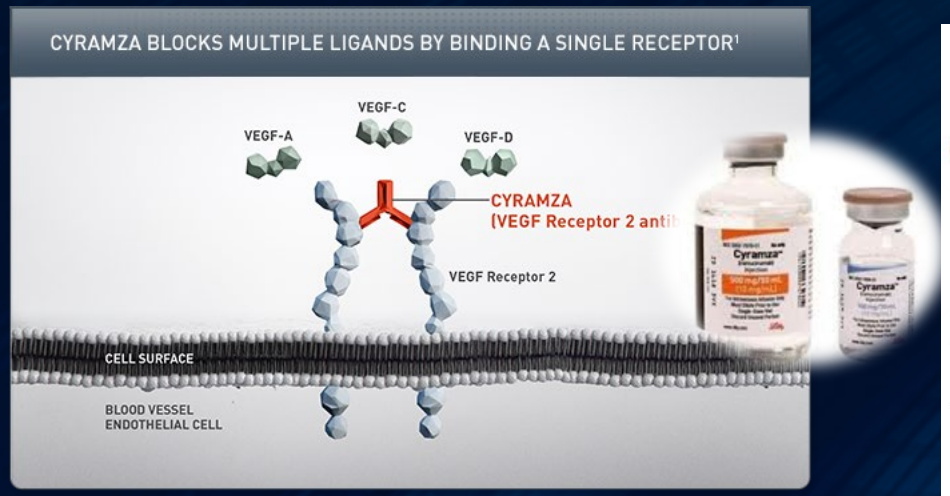
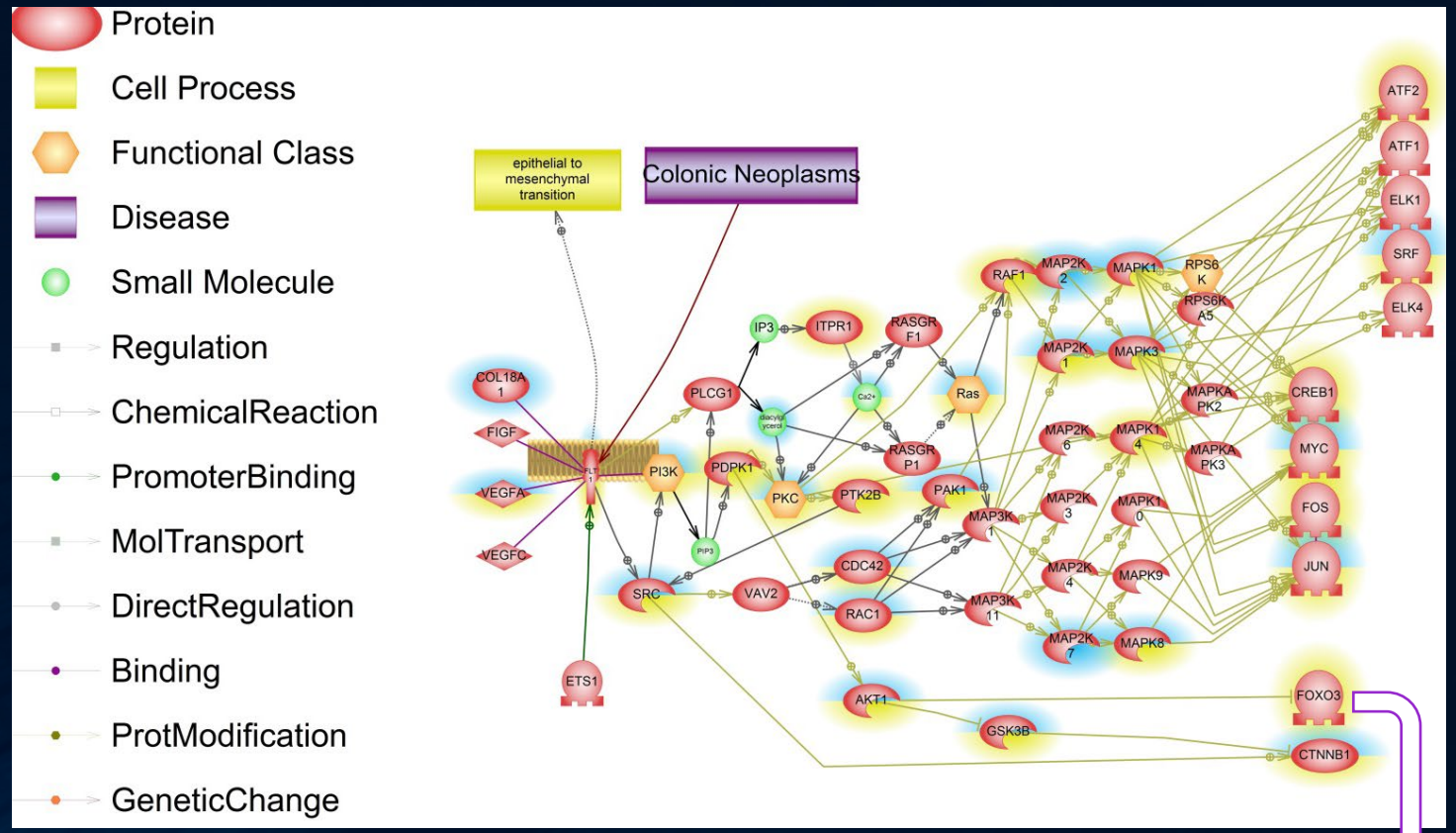
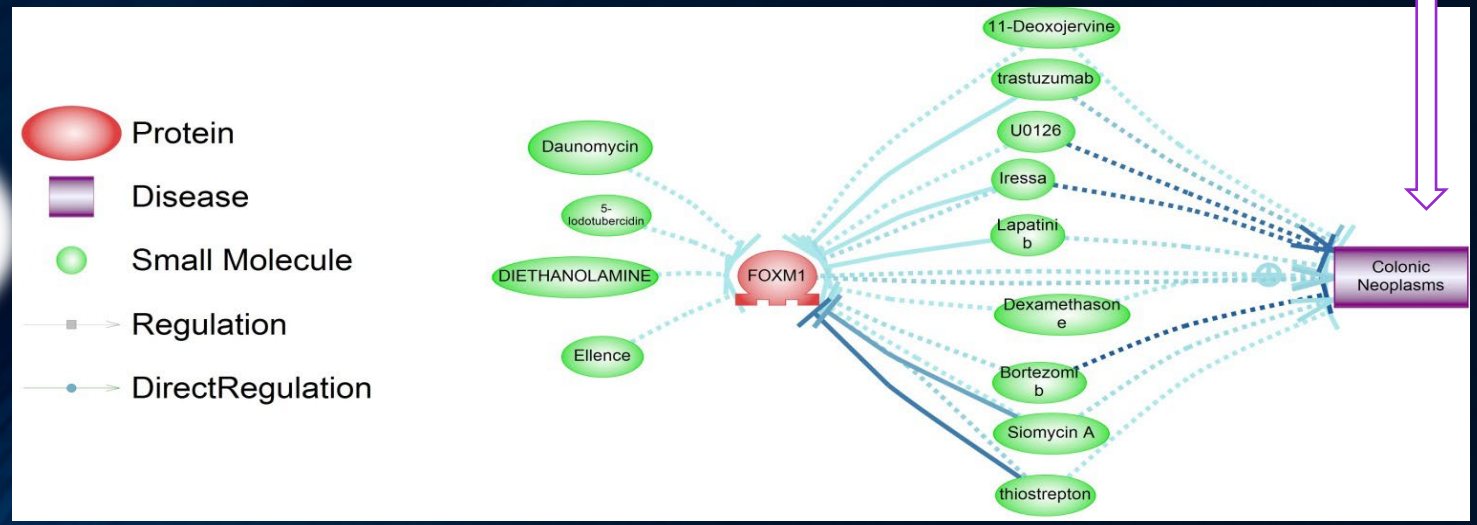
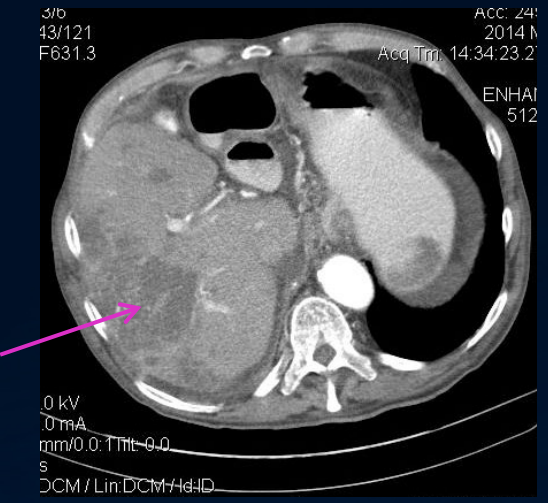


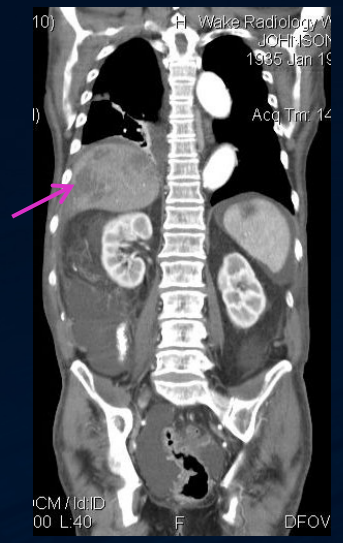
Diagram is not drawn to scale.





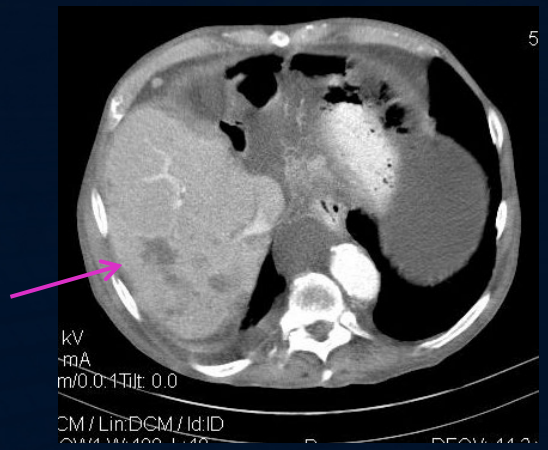


10-march-2014
Transverse CT/Scan

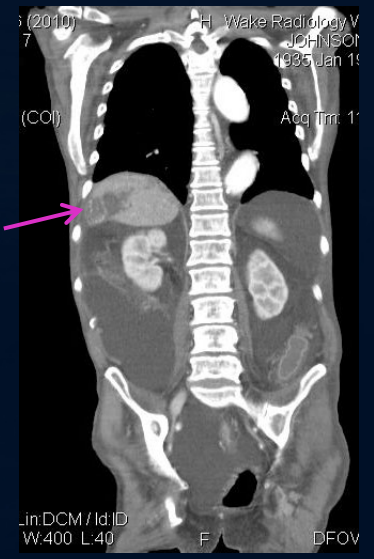


10-march-2014
Coronal CT/Scan

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

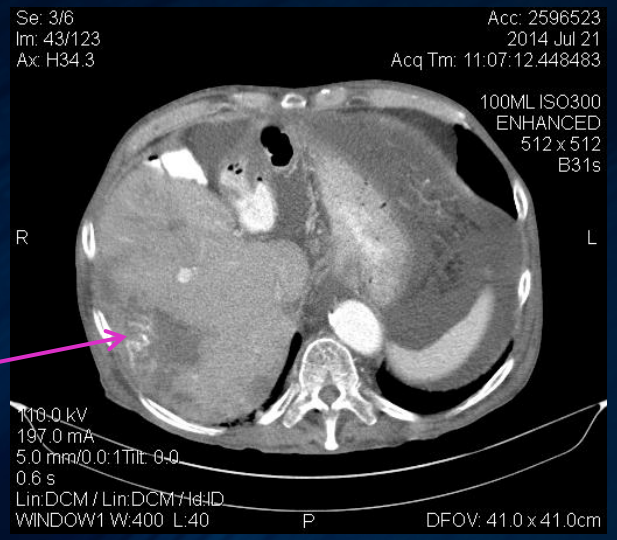


19-may-2014
Transverse CT/Scan

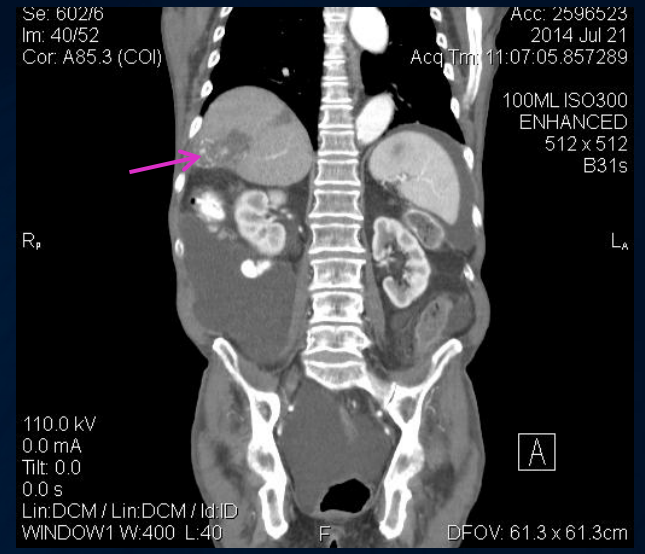


19-may-2014
Coronal CT/Scan

After 2 months off chemotherapy Scan shows no increase in number of mets 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

- ✓ Every patient with metastatic cancer should have gene expression profiling performed on their tumor cells to allow the science to dictate which proliferation pathways to block with molecularly targeted drugs to force apoptosis of their cancer cells, to look at the blueprint of the tumor cells before selecting treatment – as opposed to using standard of care cytotoxic drugs based on treating a heterogeneous population of patients with the same cancer without having looked at the tumor blueprint, relying on physician preferences created by guideline committees ("blindfold and pin the tail on the donkey")!

- ✓ In patients with metastasis or who exhausted all the treatment options, gene expression profiling (GEP) changes the prognosis and management for the majority of patients.

- ✓ Patients for whom the GEP test was performed had longer median survival than that historically reported for patients with the same diagnosis and stage of disease.

- ✓ GEP is projected not only to increase overall survival, but also decrease toxicity of treatment, and improve quality of life.