

How Science Conducted in the Lab is Changing the Structure of Clinical Trials

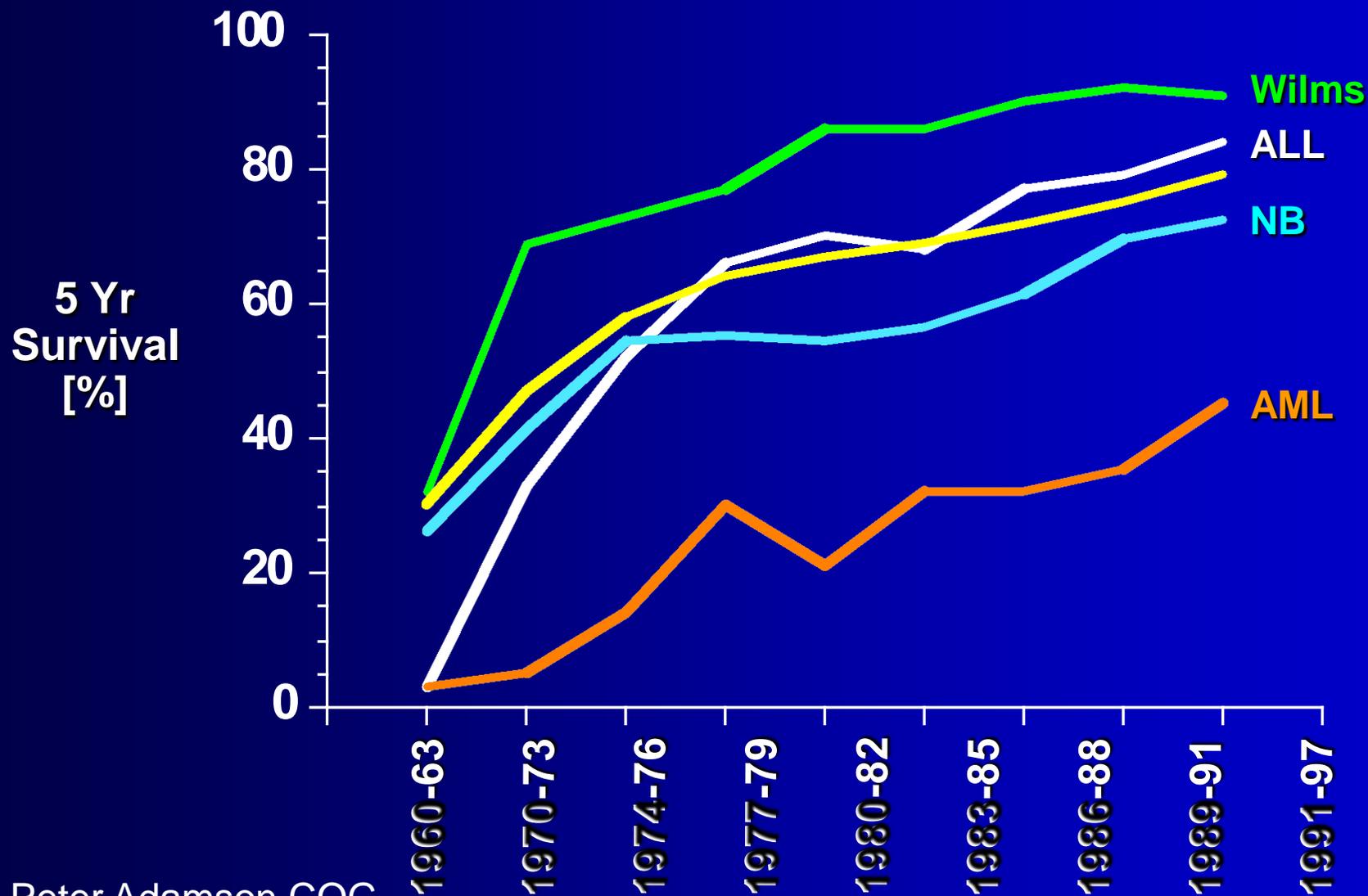
Javed Khan, M.D.
Pediatric Oncology Branch
Center for Cancer Research
National Cancer Institute

Sept 21, 2011

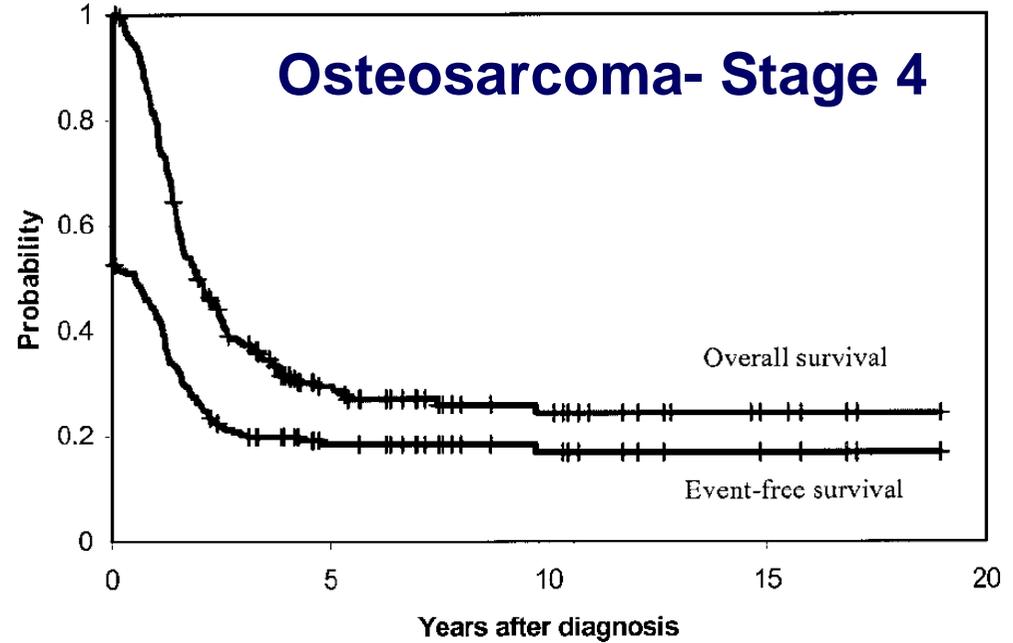
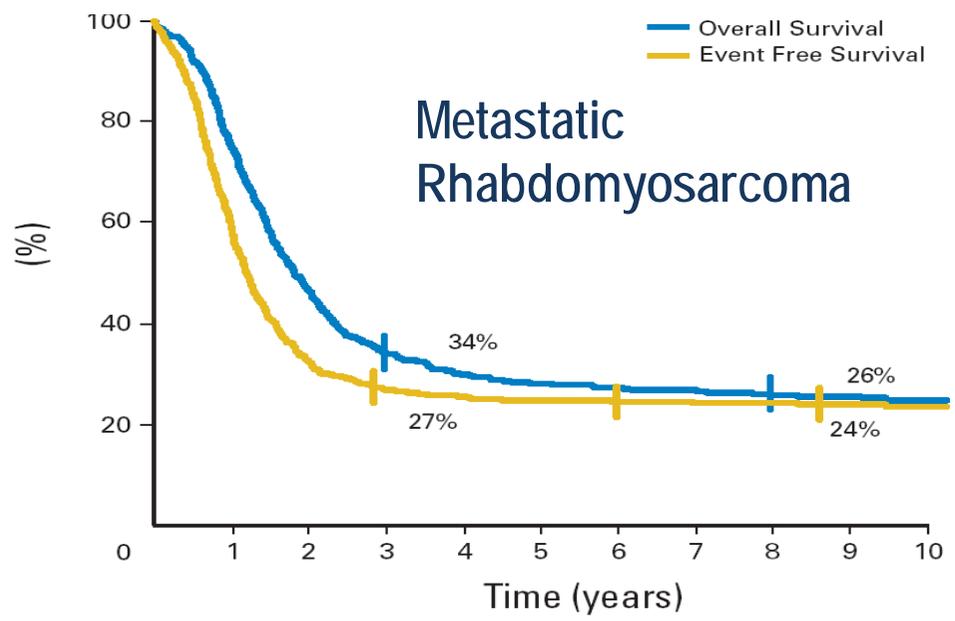
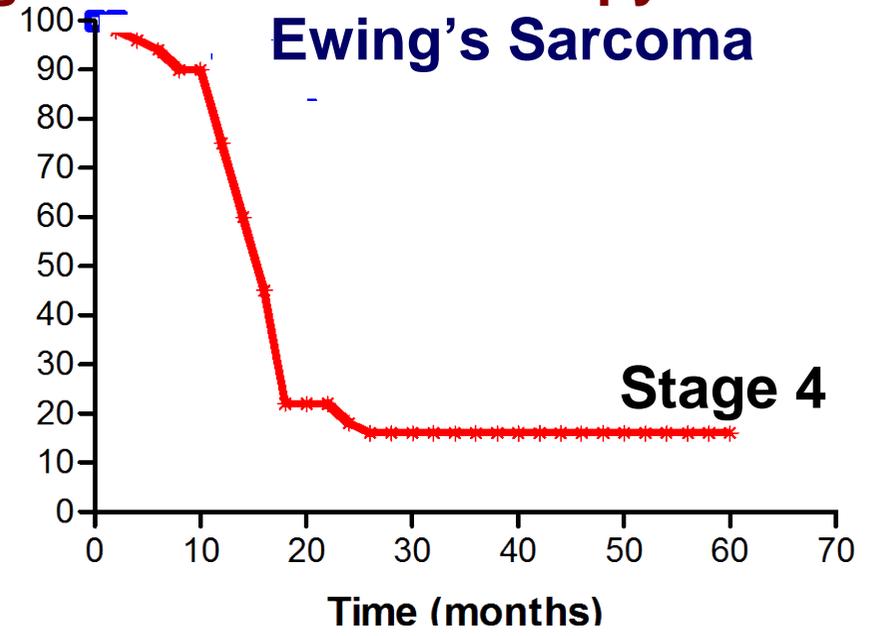
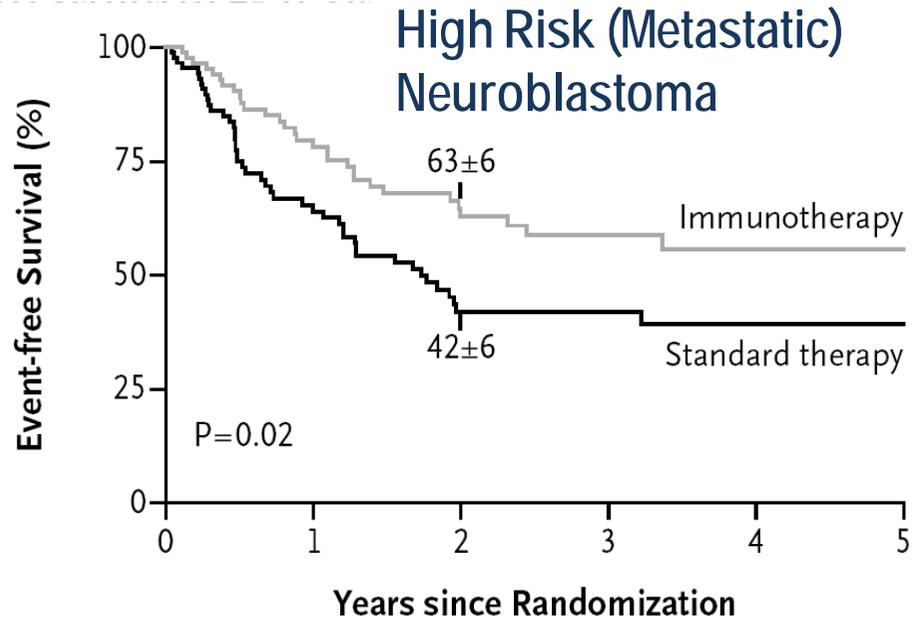
*58th Meeting of the National Cancer Institute
Director's Consumer Liaison Group (DCLG)*



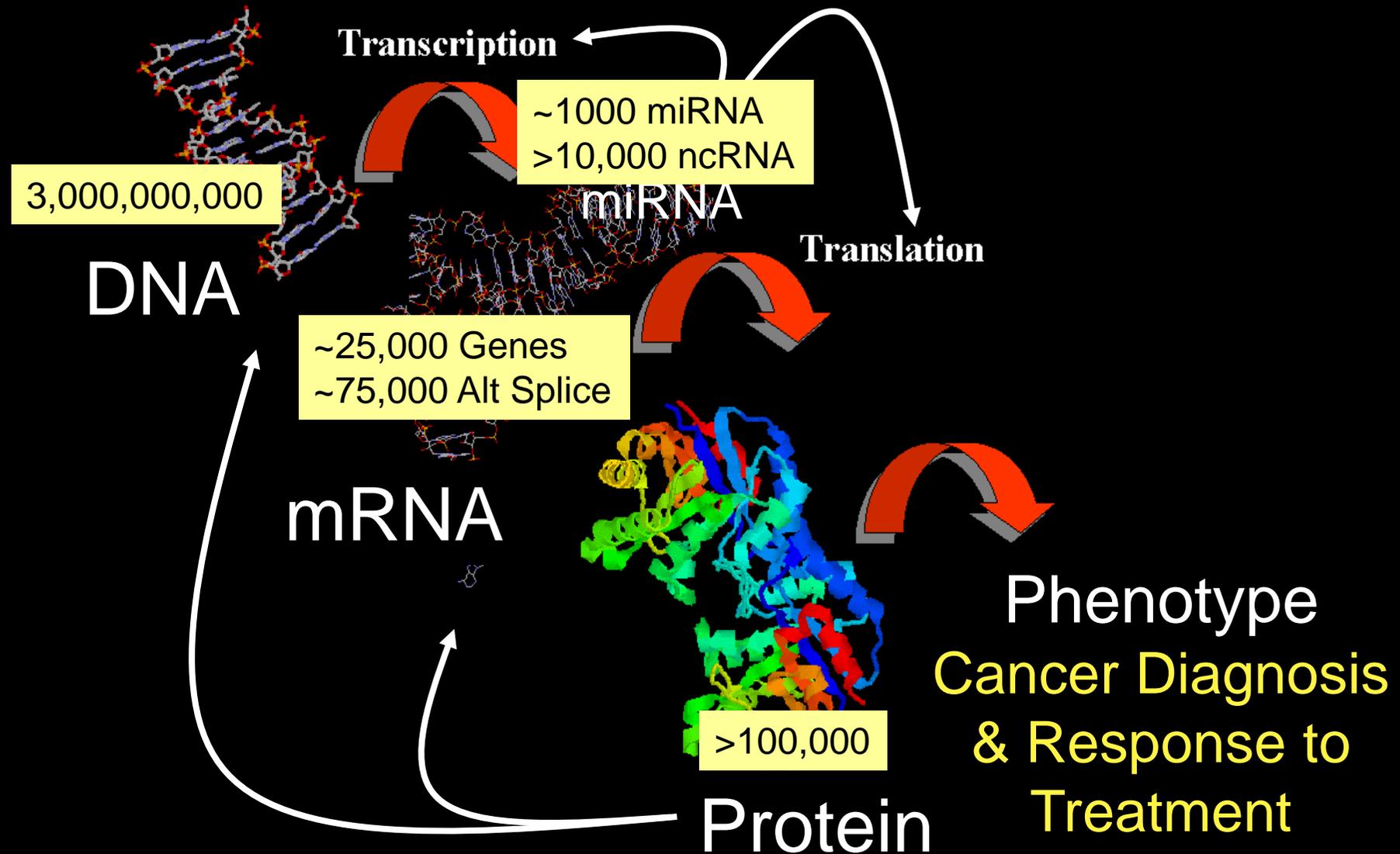
Overall Childhood Cancer Survival Has Improved Over 3 Decades



Cure rates for pediatric patients with metastatic and recurrent cancers remain poor despite decades of aggressive clinical therapy trials



Importance of Genomics: Biology is driven by the simultaneous expression of large numbers of genes acting in concert



Outline

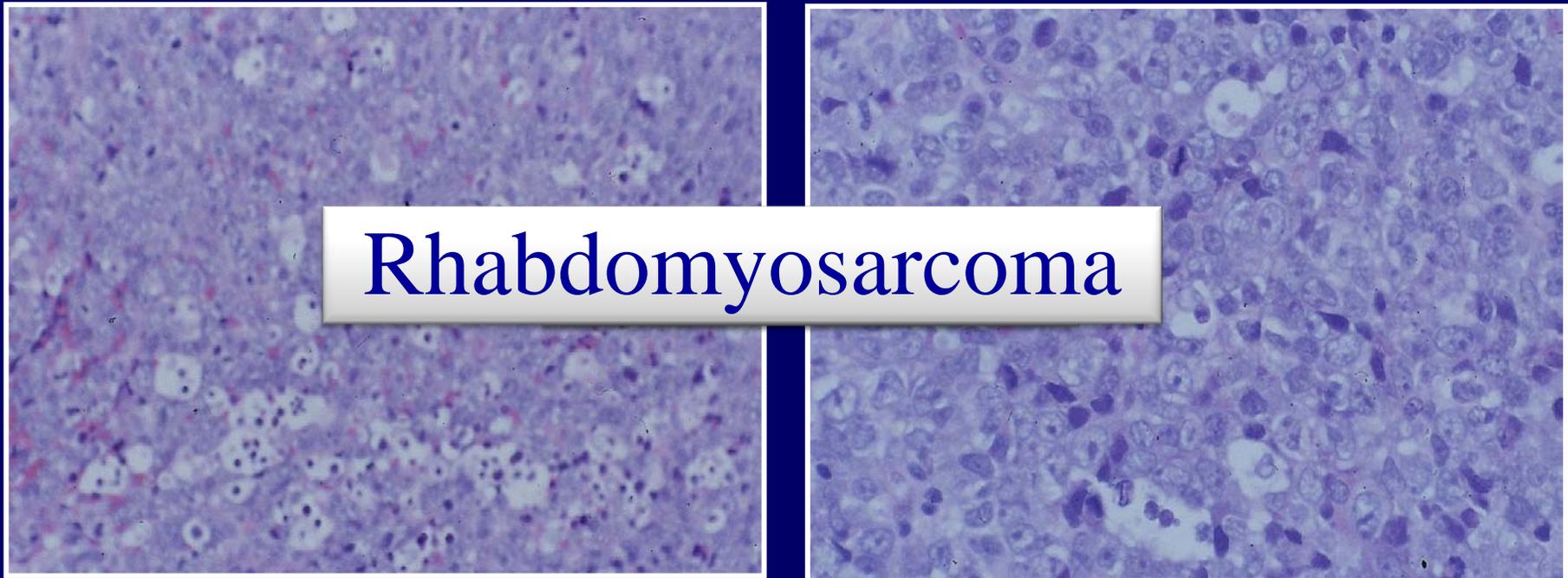
1. mRNA Diagnostic and Prognostic Biomarkers
2. Next Generation Sequencing (NGS)
3. Using Genomics to Personalize Therapy

Clinical Vignette-1

- **5 year old male referred to NCI for second opinion**
- **Injury to R groin/inguinal while playing**
- **Rapid evolving mass**
- **Diagnosis= hematoma**
- **Treatment observation**
- **Initial resolution**

- **Several weeks later mass enlarged**
- **Suspected malignancy**
- **Biopsy performed**

Despite availability of immunohistochemistry, cytogenetics and molecular techniques, in some cases incorrect diagnoses are made: Can genomics be used for better diagnostics, biology, targets, novel therapies ?



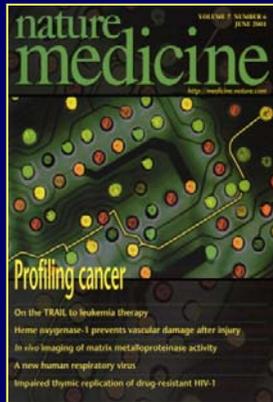
Small Round Blue Cell Tumor (SRBCT)
Lymphoma, Rhabdomyosarcoma (RMS)
Ewing's Sarcoma (EWS), Neuroblastoma (NB)

mRNA Diagnostic Biomarkers

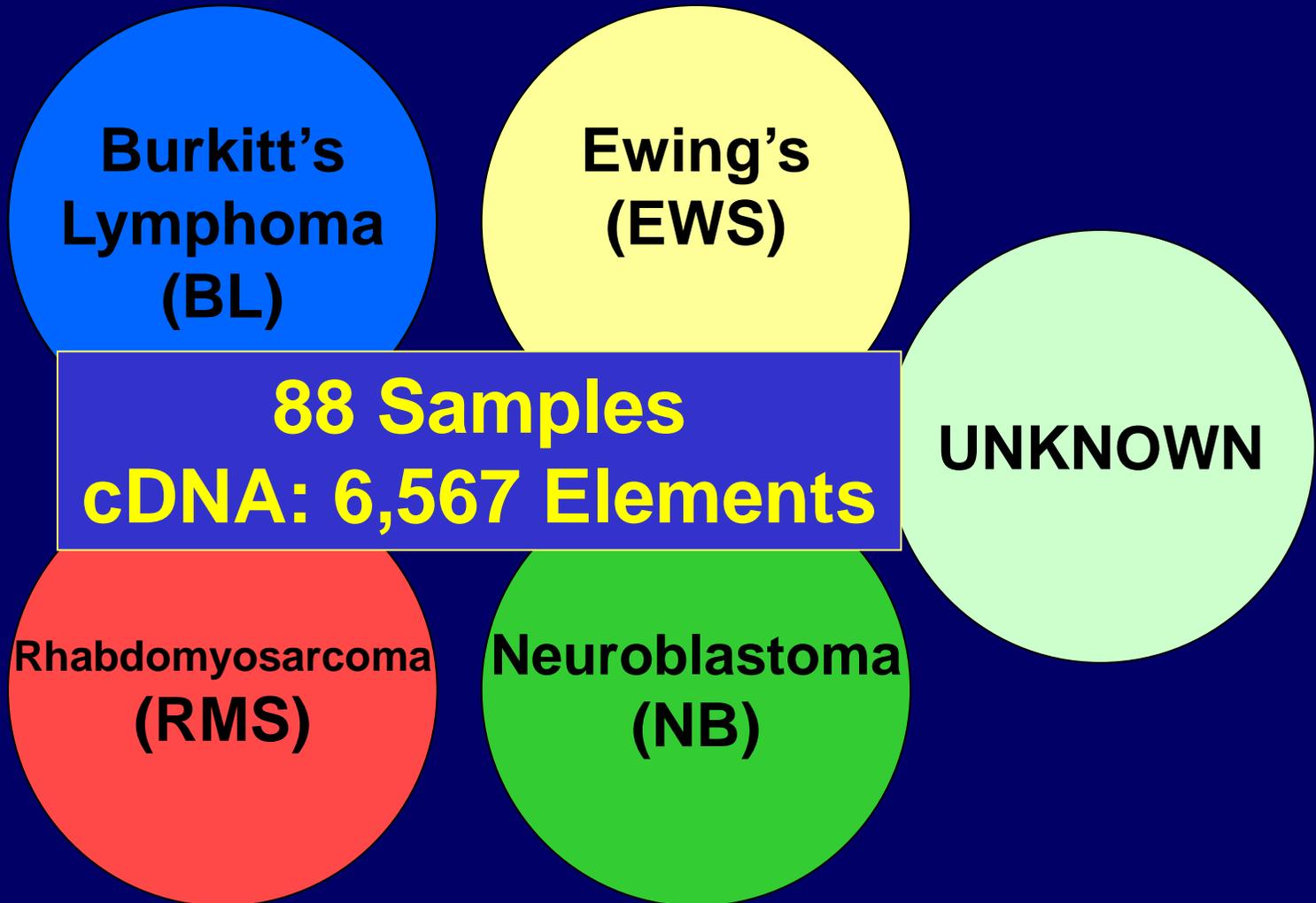
Small Round Blue Cell Tumors (SRBCT)



Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks



Khan et al., 7, 673-9, 2001



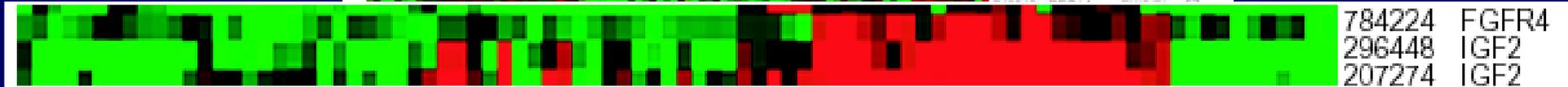
Artificial Neural Networks Analysis of Microarray Gene Expression Data Identified 96 Genes to Diagnose the SRBCTs



Image Id	Gene	Class	Rank
587708	F1FR1	NOT BL	32
563673	ATQ1	NOT BL	34
504791	GSTA4	NOT BL	59
364934	DAPK1	EWS*	82
52076	NOE1	EWS*	19
80358	SELENBP1	EWS*	63
1473131	ILE2	EWS*	35
368702	PTPN13	EWS*	15
814260	FVT1	EWS*	75

Identified 41 genes
 not previously reported
 to be expressed in SRBCTs
 -Novel diagnostic biomarkers
 -Biology
 -Candidate therapeutic targets

244618	ES1	RMS*	7
245330	IGF2	RMS*	46
42558	GATM	RMS*	77
293500	EST	RMS*	69
417226	MYC	EWS/BL	37
812965	MYC	EWS/BL	39
714453	IL4R	RMS/BL	24
824602	IFI16	EWS/BL	45



50903	F1FR1	BL	71
814526	HSRNASEBRMS/BL	78	



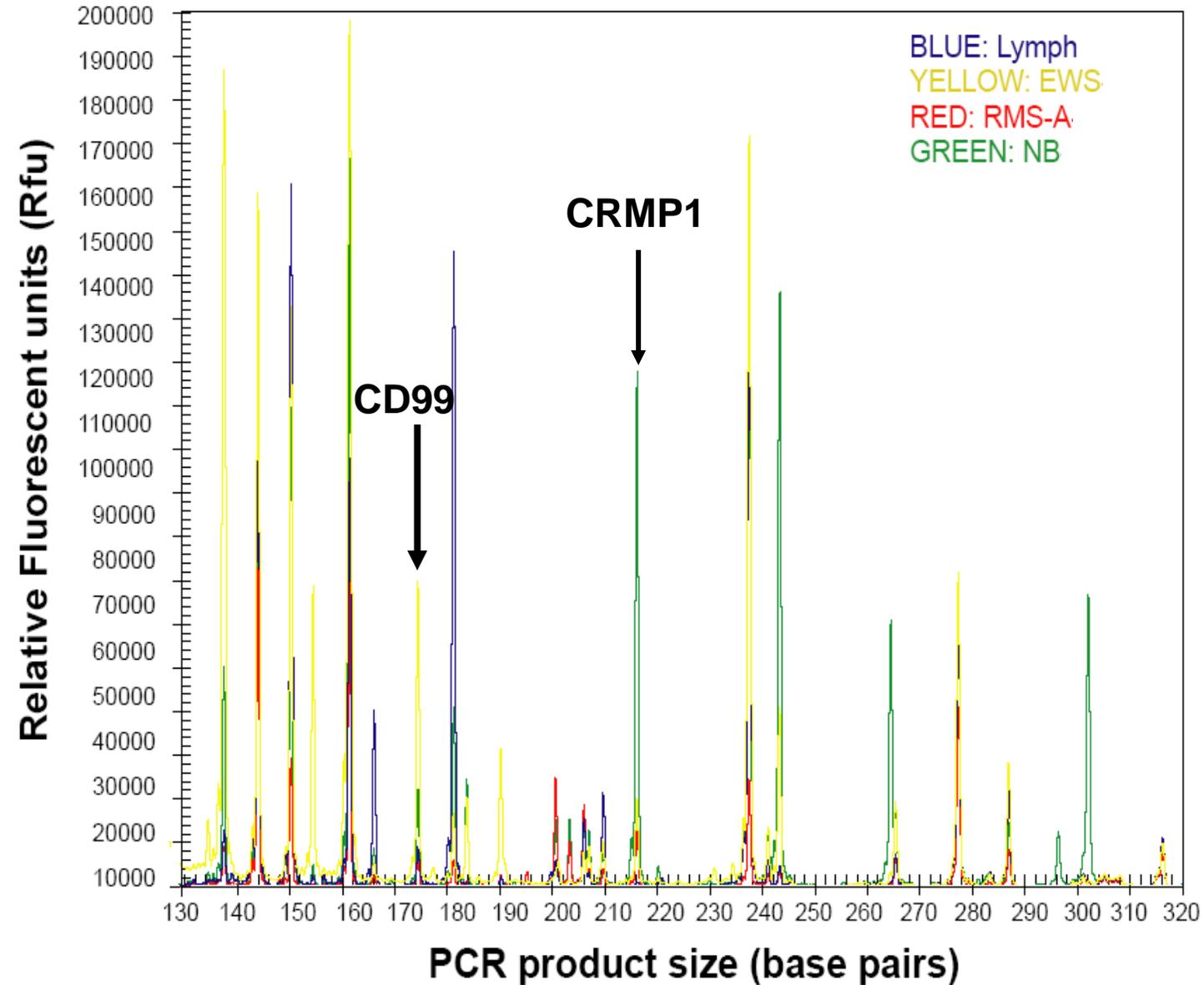
Translation of Microarrays to Clinic: Biomarkers for Diagnostics of Small Round Blue Cell Tumors (SRBCTs)

- Multiplex RT-PCR- Collaboration Althea Technologies*
- Top 34 ANN ranked SRBCT (Khan et al 2001 Nat Med) + 5 genes from NB vs. Normal tissue study (Son, Khan et al Genome Research 2005)
- 96 Independent SRBCT Samples (RNA 25 nanograms)

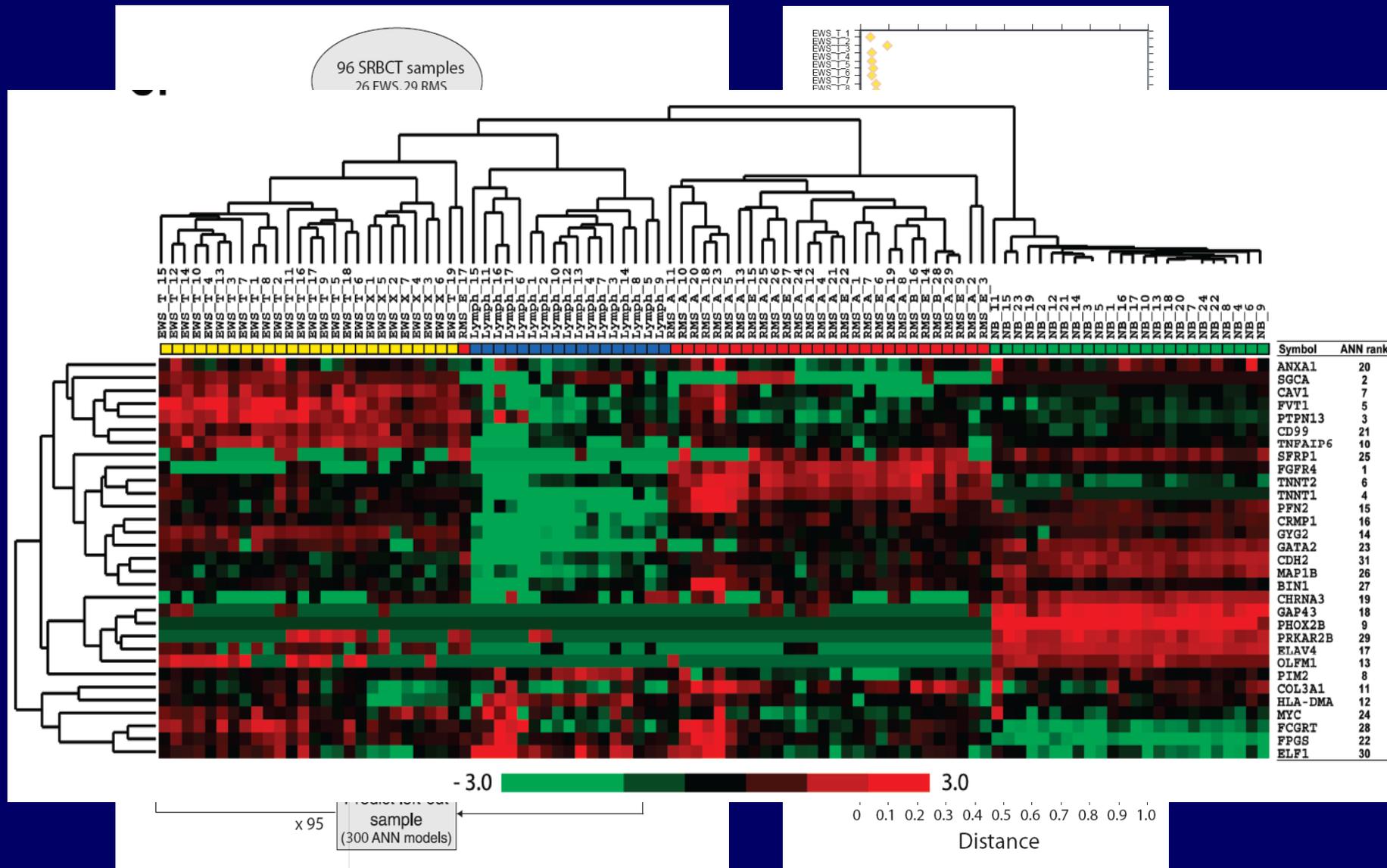
J Mol Diagn. 2007, 9:80-88.

Qing-Rong Chen, Jun Wei and Young Song

Diagnosis of SRBCT using Multiplex PCR



Diagnosis of SRBCT using Multiplex PCR- and Artificial Neural Networks



Diagnosis of SRBCT using Multiplex PCR- and Artificial Neural Networks

**Ready for Clinic-Aid to Pathologists
for Rapid Diagnosis from Needle
Biopsies**

EWS (n=26)	100	98.6
RMS (n=29)	96.6	100
NB (n=24)	100	100
Lymph (n=17)	100	100

**Pre-IDE meeting with FDA
Validation & Testing Under Way
Modified for FFPE**

x 95

sample
(300 ANN models)

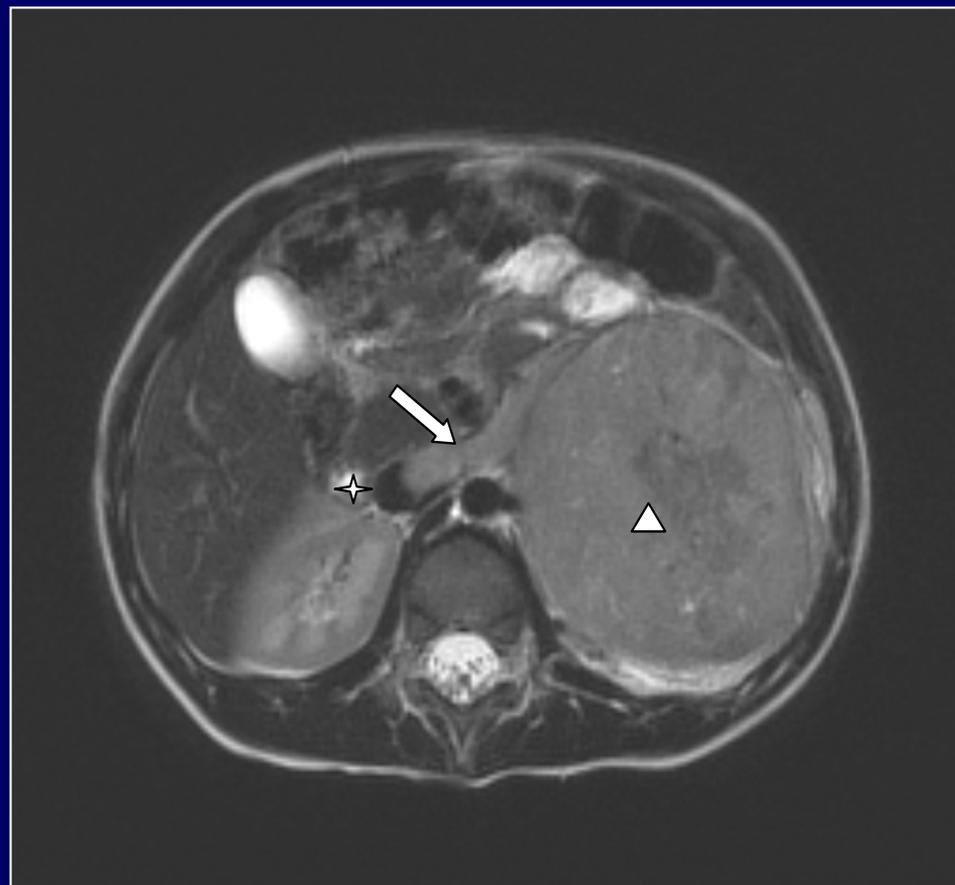
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0

Distance

Clinical Vignette-2

- **4.5 year old female 2nd ? Diagnosis**
- **6-week history of weight loss, reduced appetite, fever, abdominal pain**
- **On examination left sided abdominal mass**

**MRI: 9 x 8 x 9 cm mass in upper pole left kidney, tumor
in Left renal vein and inferior vena cava**

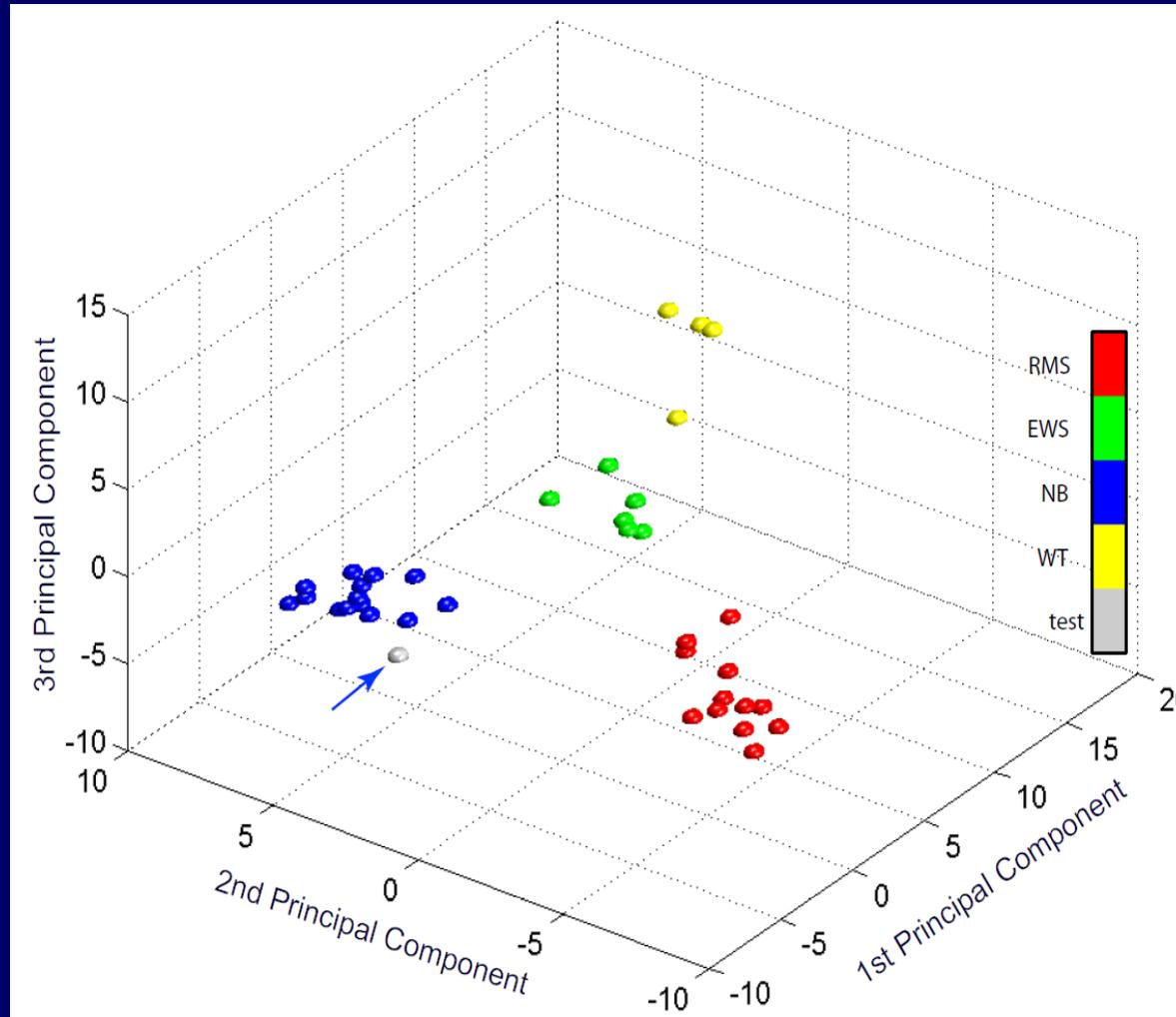


Clinical Course

- **No biopsy-risk of bleeding**
- **Urine catecholamines negative**
- **MIBG scan negative**
- **Diagnosed as Wilms (nephroblastoma)**
- **Chemotherapy started**

- **6 weeks radical tumor nephrectomy**
- **Histology ?undifferentiated neuroblastoma**
- **?Diagnosis**

Microarray (Affymetrix Plus2 (84 genes): Confirms Diagnosis of Neuroblastoma and not Wilms Tumor



Summary for Genome Based Diagnostic Biomarkers in Pediatrics

- Signatures exist for highly accurate diagnostic biomarkers that can be utilized in the clinic that can aid clinicians and pathologists to manage patients with pediatric cancers.
- However because of the rarity of pediatric cancers and therefore low profit margins it is a significant challenge to arouse interest in industry to develop and market these assays.
- Not all pathologists are informed of these methods
- Our group and others will continue to pursue & develop these biomarkers and raise interest in industry and among pathologists

mRNA Prognostic Biomarkers

Neuroblastoma

Prediction of Clinical Outcome Using Gene Expression Profiling and Artificial Neural Networks for Patients with Neuroblastoma

Jun S. Wei,¹ Braden T. Greer,¹ Frank Westermann,² Seth M. Steinberg,³ Chang-Gue Son,^{1,4} Qing-Rong Chen,¹ Craig C. Whiteford,¹ Sven Bilke,¹ Alexei L. Krasnoselsky,¹ Nicola Cenacchi,¹ Daniel Catchpoole,⁵ Frank Berthold,⁶ Manfred Schwab,² and Javed Khan¹

October 1, 2004
Volume 64
Number 19
Pages 6835–7182

Cancer Research

The flowchart illustrates the study methodology. It starts with 56 NB Samples (34 Alive, 22 Deceased). 35 NB Samples are selected for training (19 Alive, 17 Deceased), and 21 NB Samples are reserved for testing (11 Alive, 10 Deceased). The process involves Principal Components Analysis (25 x 15), Training ANNs (800 Models), and Gene Minimization. This leads to 19 Genes (35 x 13), which are used to Train ANNs (800 Models) and Predict 21 Samples. The final output is a prediction of clinical outcome.

Men1-Mediated Tumorigenesis in the Absence of Chromosome Instability

Control of Bleomycin-Induced Fibrosis by H2-Ea

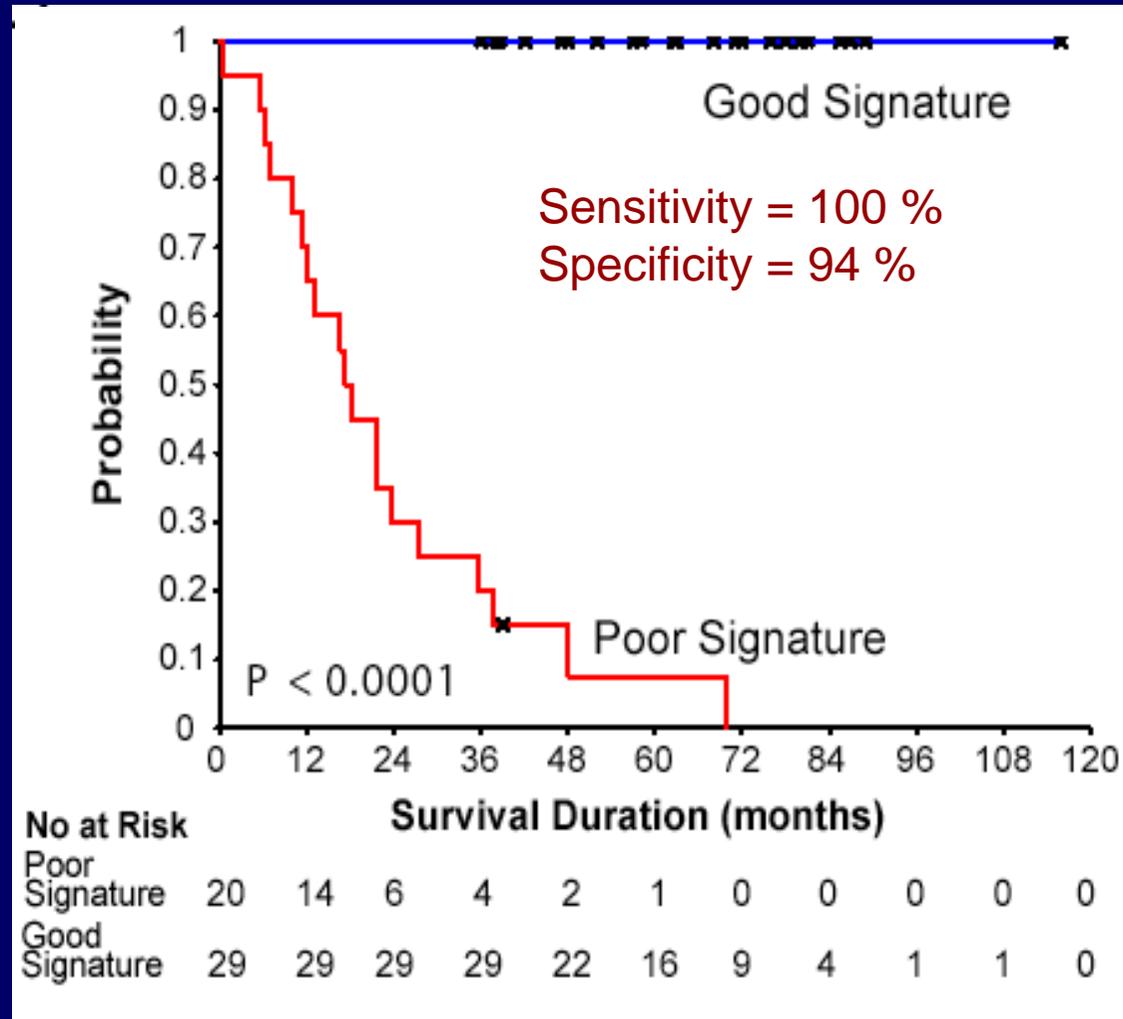
Predicting Prognosis With Artificial Neural Networks

www.aacrjournals.org American Association for Cancer Research

Can we predict outcome of individual patients based solely on gene expression profiles?

- 42, 000 cDNA Microarray
- 49 Patients: (19 Dead of Disease, 30 disease free >3yrs)
- Use Artificial Neural Networks (ANN) to Predict Outcome and Identify Prognostic Signature

Identified 19/42,000 that resulted in the least misclassification rate:
 Survival probability using top 19 genes



Summary and Challenges of using mRNA Prognosis Study

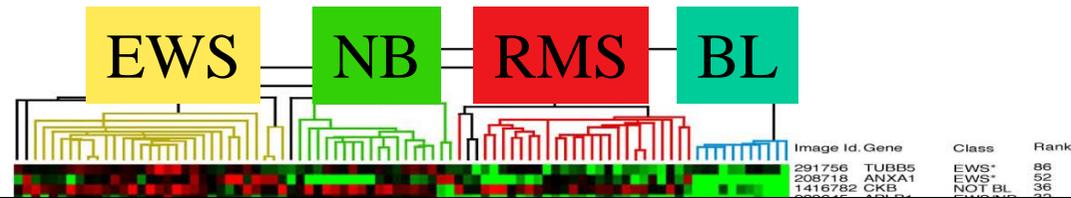
1. Gene expression profiles contain prognostic information in the pre-treatment samples
2. Personalized medicine, predicting outcome of individual tumors, may be possible
3. Even if we can predict outcome for high risk patients there is a paucity of validated targets and drugs
4. Pilot Study with small numbers-requires independent validation study. TARGET-NBL (Therapeutically Applicable Research to Generate Effective Treatments)
5. However multiple Neuroblastoma prognostic gene expression signatures published very little overlap-prospective trials required
6. mRNA expression signatures fraught with inherent problems (handling, instability, heterogeneity, retrospective)

Applying Genomics to Identify Novel Targets for Therapy

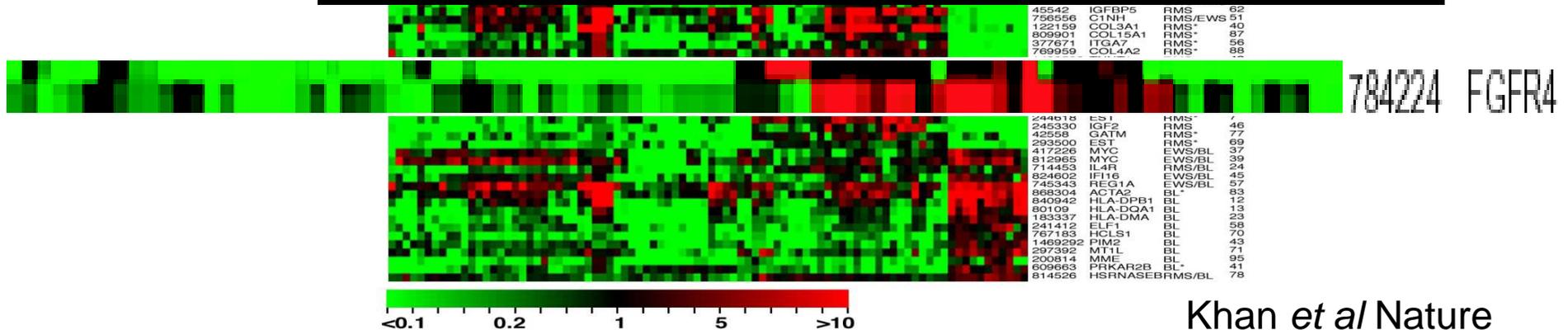
Rhabdomyosarcoma (RMS)

- **Arises from progenitor cells of striated skeletal muscle**
- **Third most common soft tissue sarcoma in children (5% of all pediatric tumors)**
- **Incidence is 4.3 cases per million children (about 350 new cases each year in US)**
- **Two major histological subtypes**
 - **Embryonal (LOH 11p15)**
 - **Alveolar (t(2;13) or t(1;13); PAX3/7-FOXO1A)**
- **Survival <30% for metastatic disease**

FGFR4 is over-expressed in RMS



- Tyrosine kinase cell surface receptor
- Over expressed in ~100% RMS
- Direct target of PAX3 and PAX3-FKHR
- Expressed during muscle development
- Induced in regenerating muscle after injury
- Not expressed in mature muscle
- Suggest possible role in myogenic stem cells
- Suggests possible oncogenic role in RMS

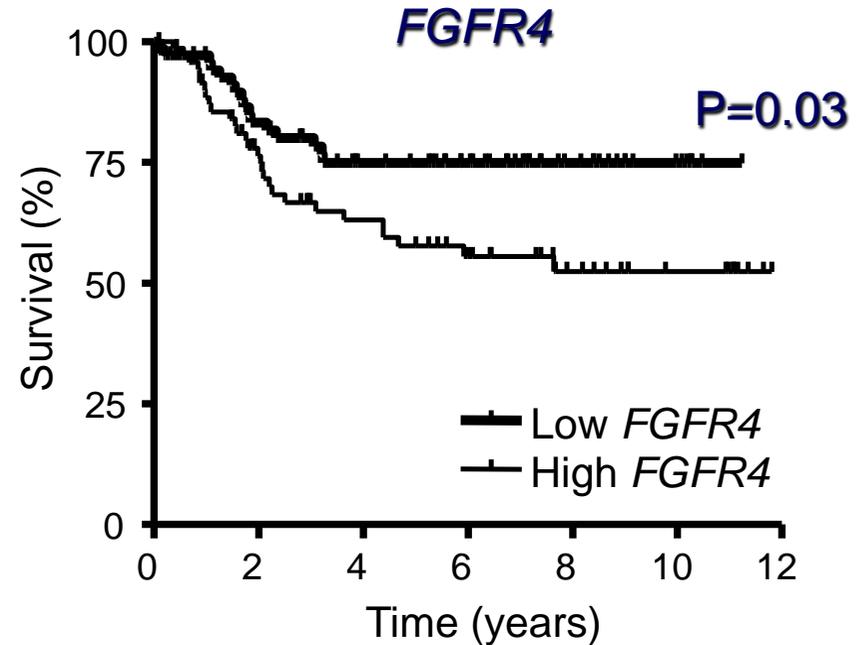
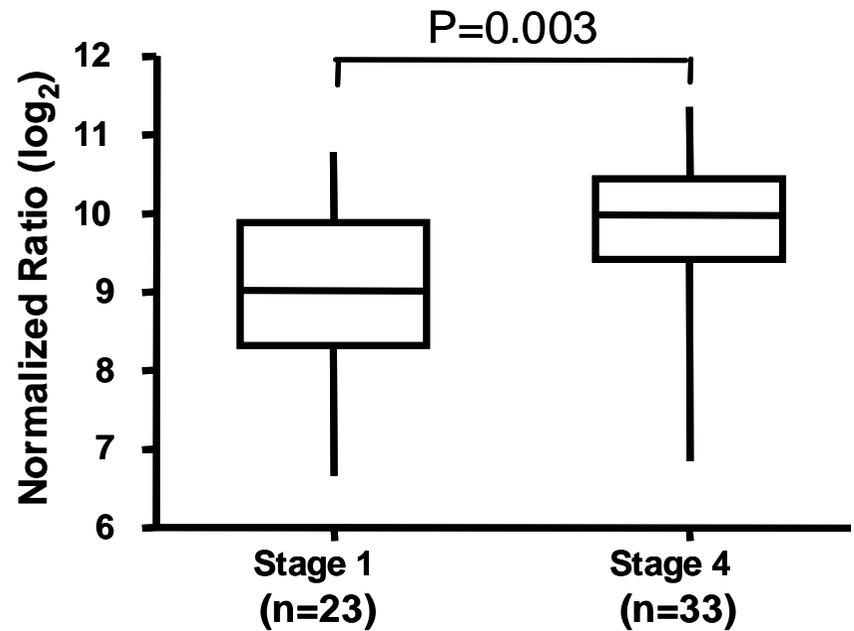


Does FGFR4 play a role in RMS biology?

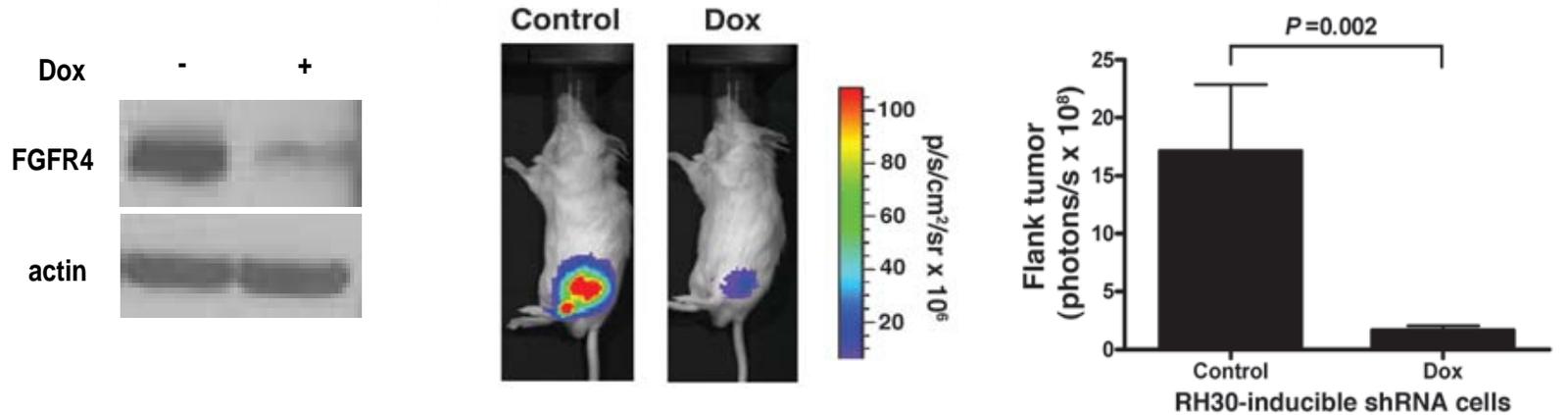
Is it activated in RMS?

Is it a good target for therapy

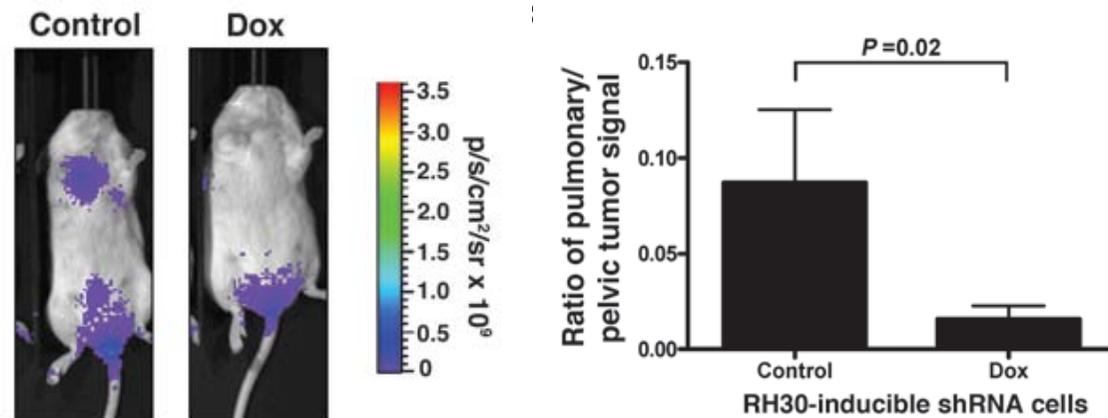
High *FGFR4* Expression Associated with Advanced Stage and Poor Survival in RMS



FGFR4 suppression by shRNA inhibit tumor growth and lung metastasis in RH30



Intra-muscular injection



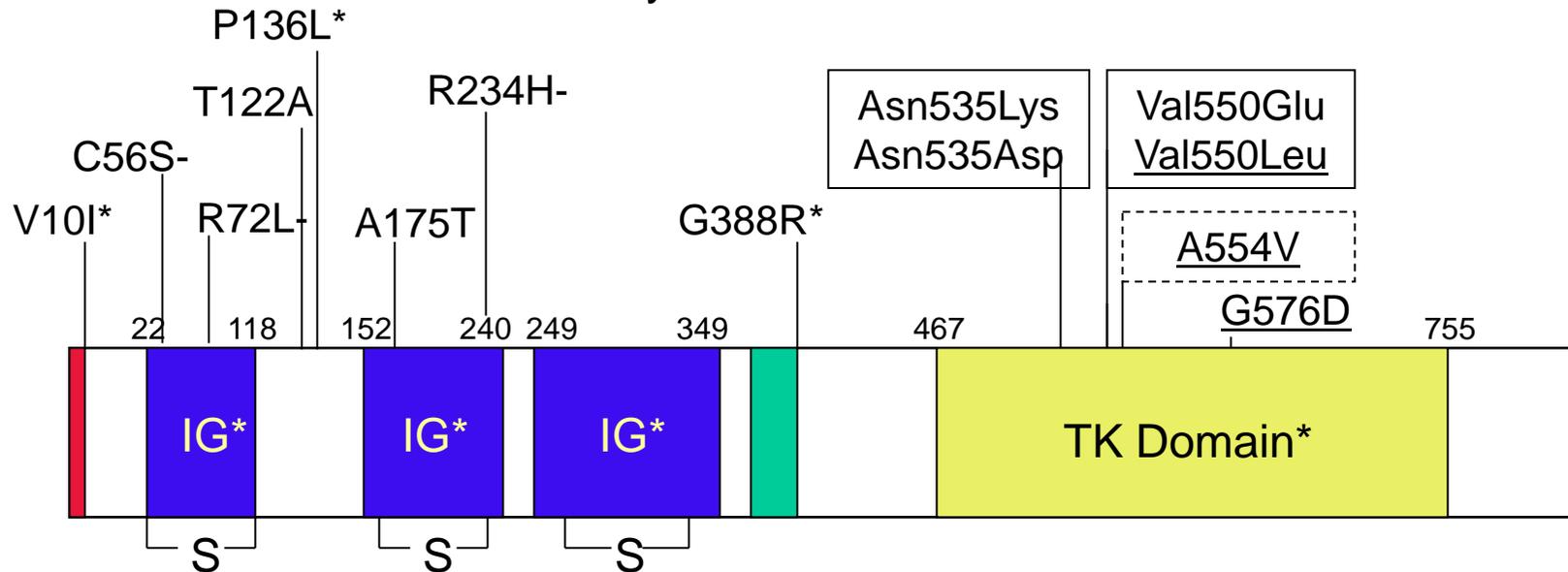
Tail vein injection

**Is FGFR4 mutated in RMS?
Are they activating mutations?**

FGFR4 Sequencing (17 coding Exons)



94 Rhabdomyosarcoma tumor samples
1030 Healthy Controls



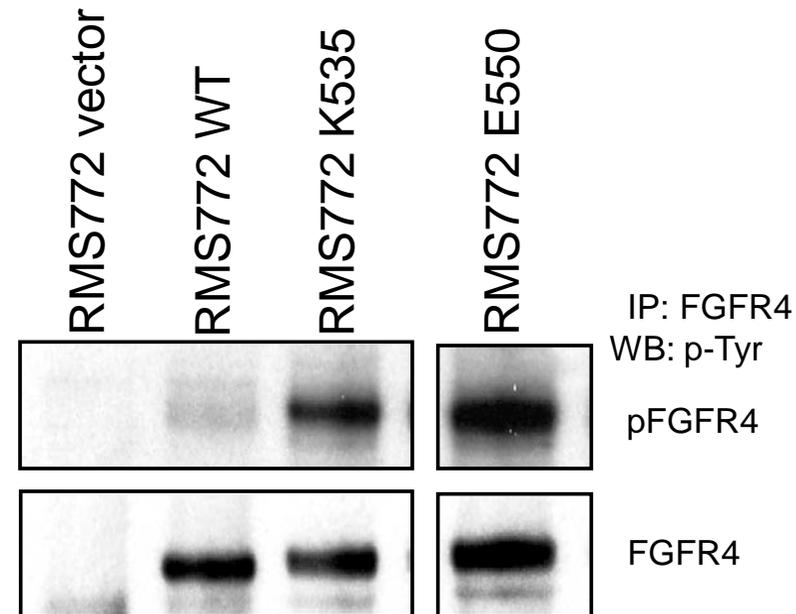
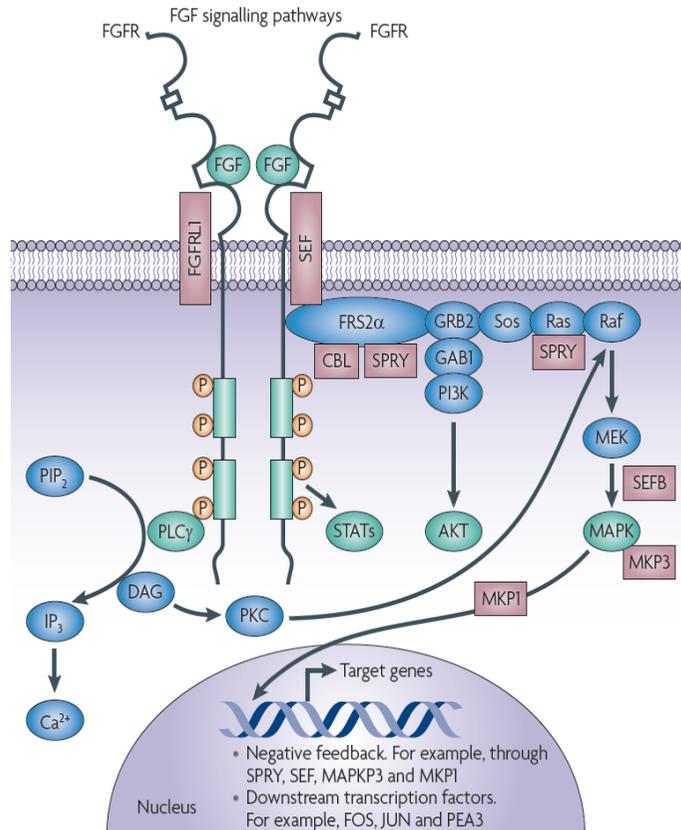
- 46 Single Nucleotide Variants
- 26 coding SNVs

- **14 missense mutations**
- **Cluster of somatic mutations in exons 12 and 13 (7.5%) not seen in 1030 healthy controls ($p=2.0 \times 10^{-7}$), dbSNP or 1000 genomes**

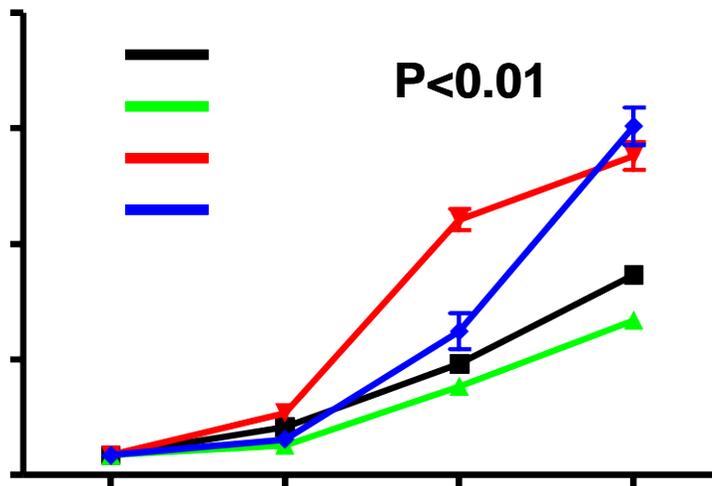
N535K
(Asparagine535Lys)
N535D
(Asn535Aspartate)
V550E
(Val550Glutamate)
V550L (Val550Leu)

Mutations lead to auto-phosphorylation of FGFR4

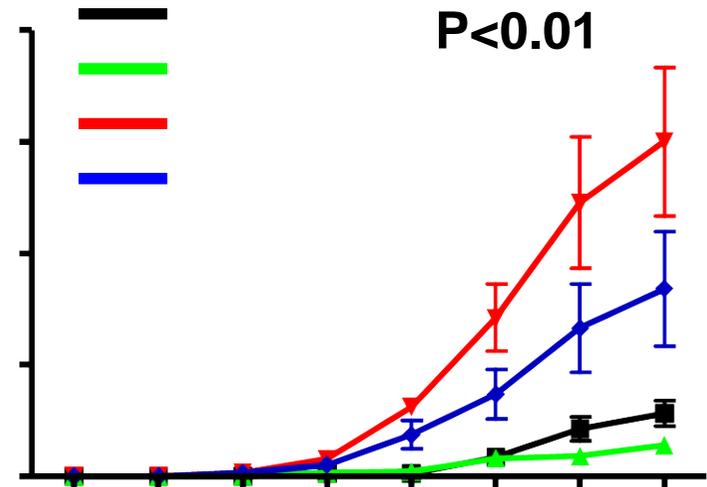
Predict Ligand Independent Activation



Increase of growth of mutants *in vitro* and *in vivo*

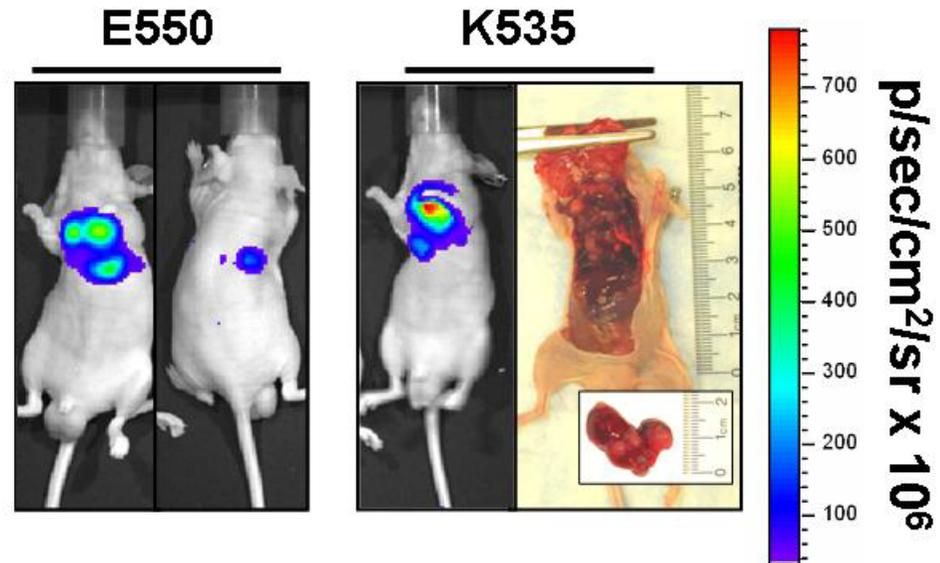
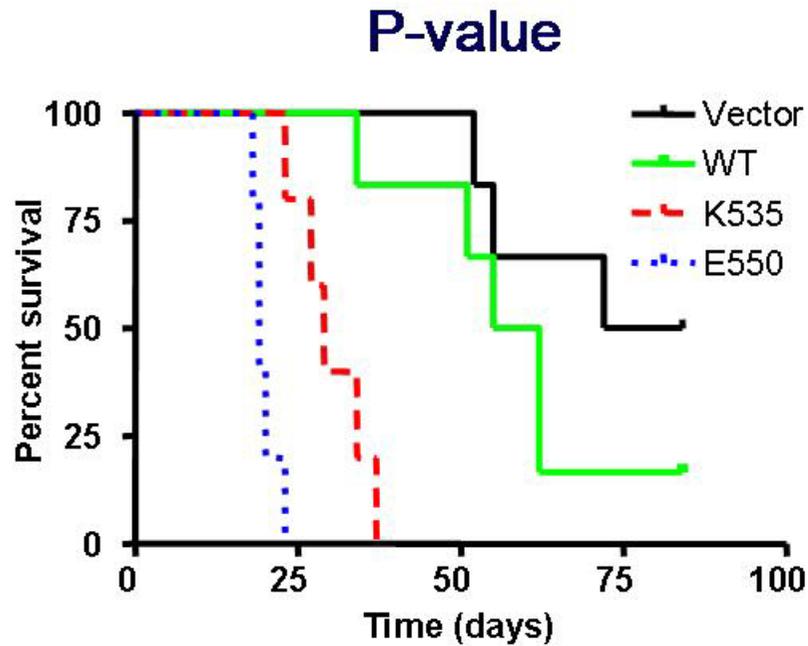


In vitro



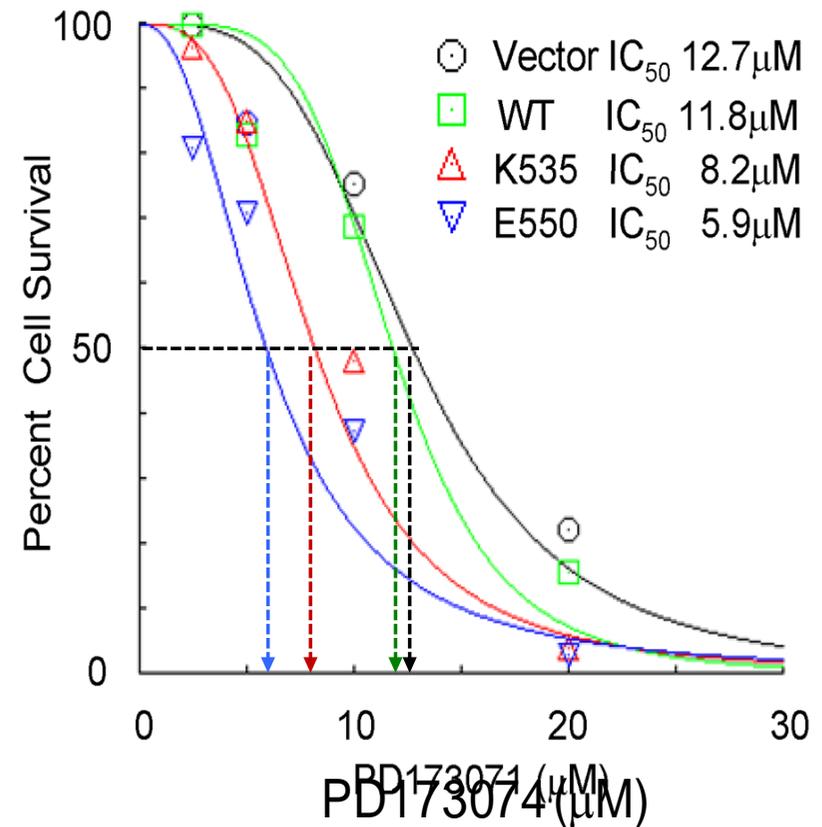
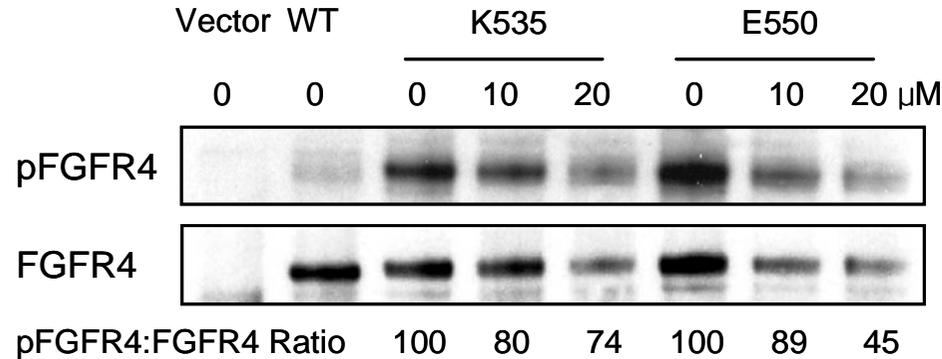
In vivo

FGFR4 mutants associated with early mortality from metastasis



Mutants Demonstrate Reduced Auto Phosphorylation of FGFR4, Increased Apoptosis & Sensitivity to and FGFR PD173074

Cells Harboring these Mutants are Oncogene Dependent

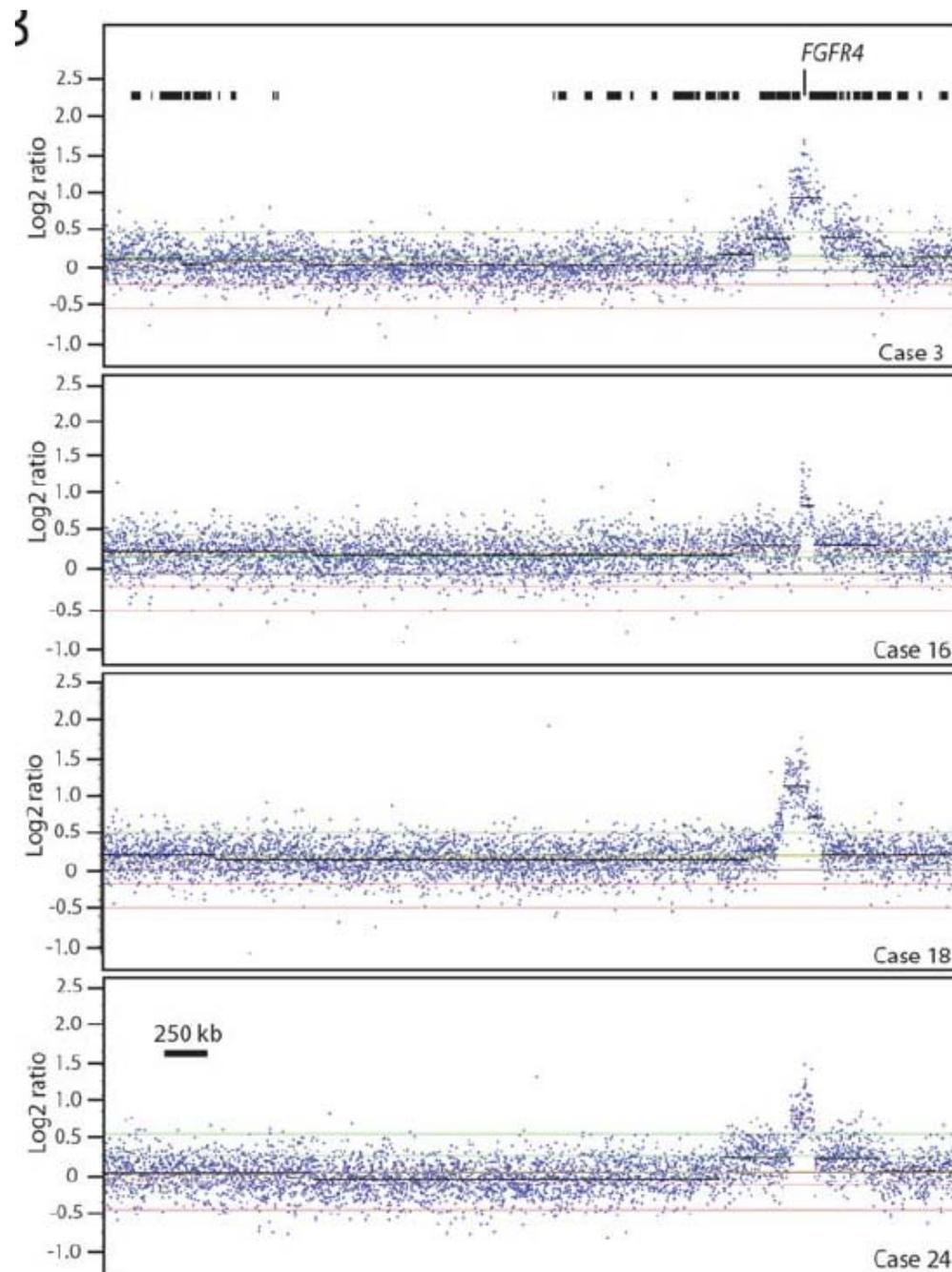


High-Resolution Array CGH Identifies Common Mechanisms that Drive Embryonal Rhabdomyosarcoma Pathogenesis

Vera Paulson,^{1,2,3} Garvin Chandler,^{1,2} Dinesh Rakheja,^{4,5} Rene L. Galindo,^{2,4,5} Kathleen Wilson,^{5,6} James F. Amatruda,^{1,2,3,7} and Scott Cameron^{1,2,3*}

FGFR4 activated in 20% with
amplification of mutant in 15% (4/26)

FGFR4 is amplified in ERMS

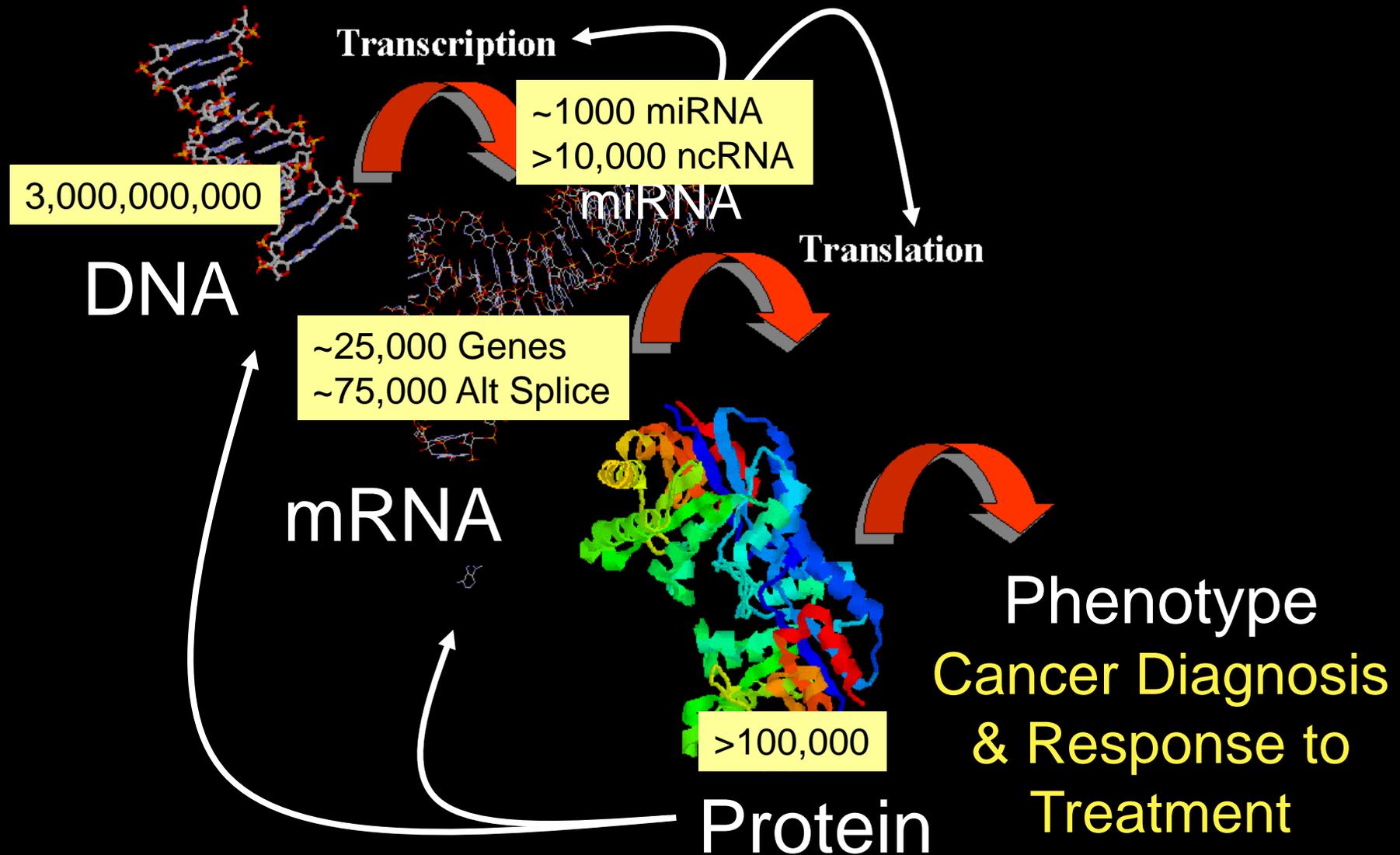


Conclusions

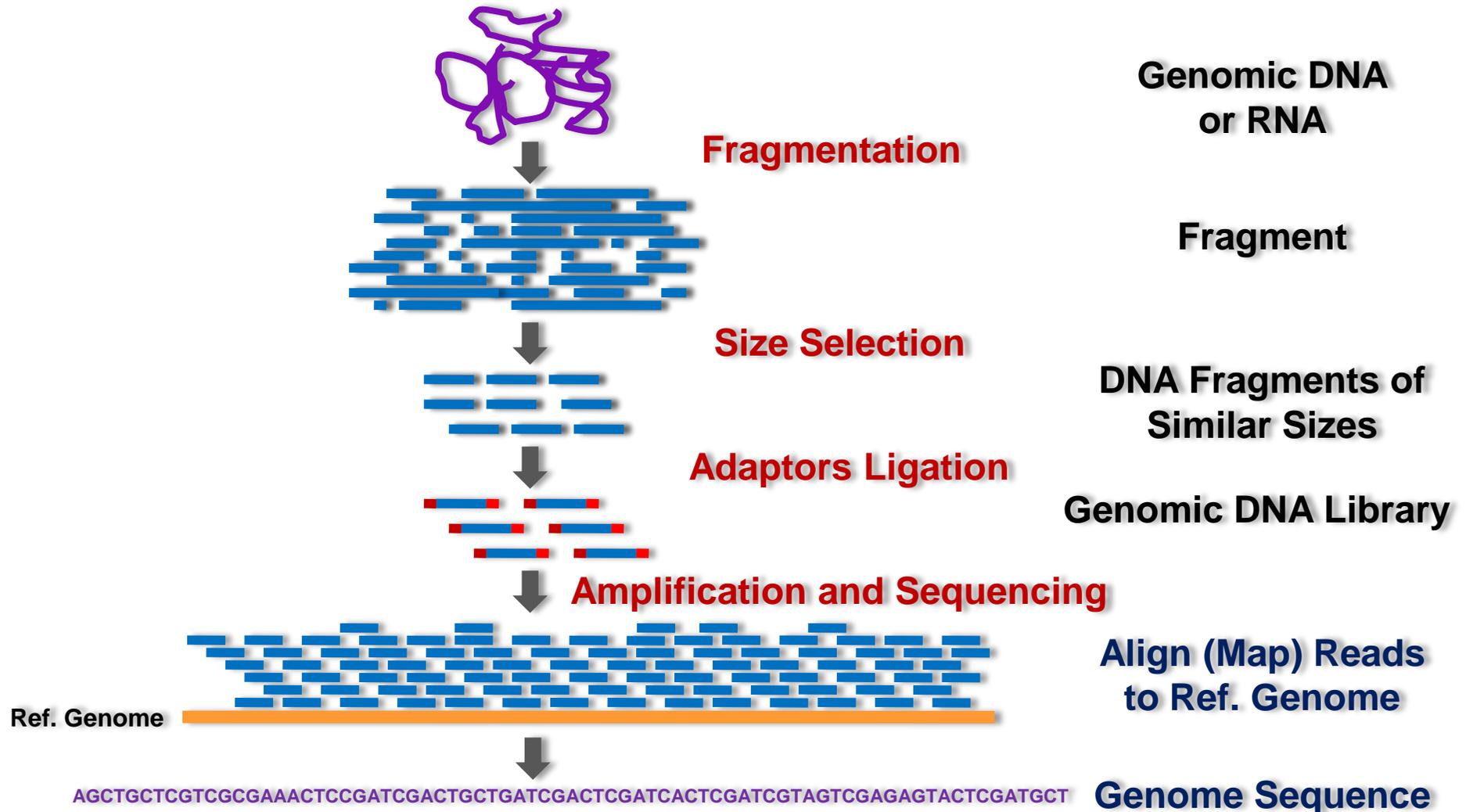
- FGFR4 is overexpressed in up to 100% of RMS
- FGFR4 is a direct target of fusion gene PAX3-FOXO1A
- Suppression of WT FGFR4 leads to reduced RMS growth and metastasis and is a good target for therapy in non mutated RMS
- Up to 20% of RMS have mutations in TK domain and/or amplification of FGFR4 (ERMS)
- Activating mutations K535 and E550 promote RMS survival and metastasis (others being tested)
- Clinical trial with inhibitors is warranted

Massively Parallel Sequencing (Next-Generation Sequencing)

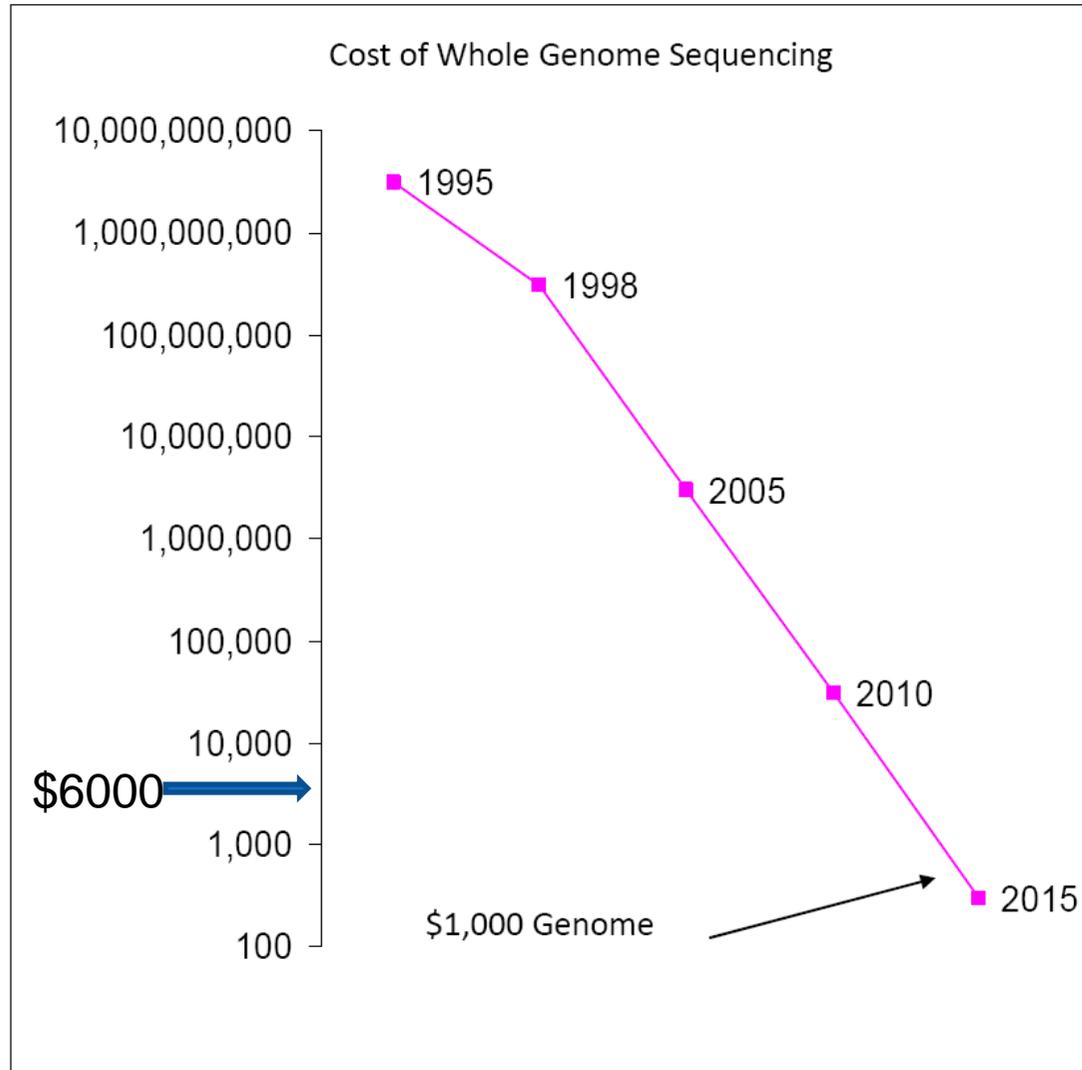
Importance of Genomics: Biology is driven by the simultaneous expression of large numbers of genes acting in concert



Massively Parallel Sequencing (Next-Generation Sequencing)



Cost of Whole Genome Sequencing Continues to Fall

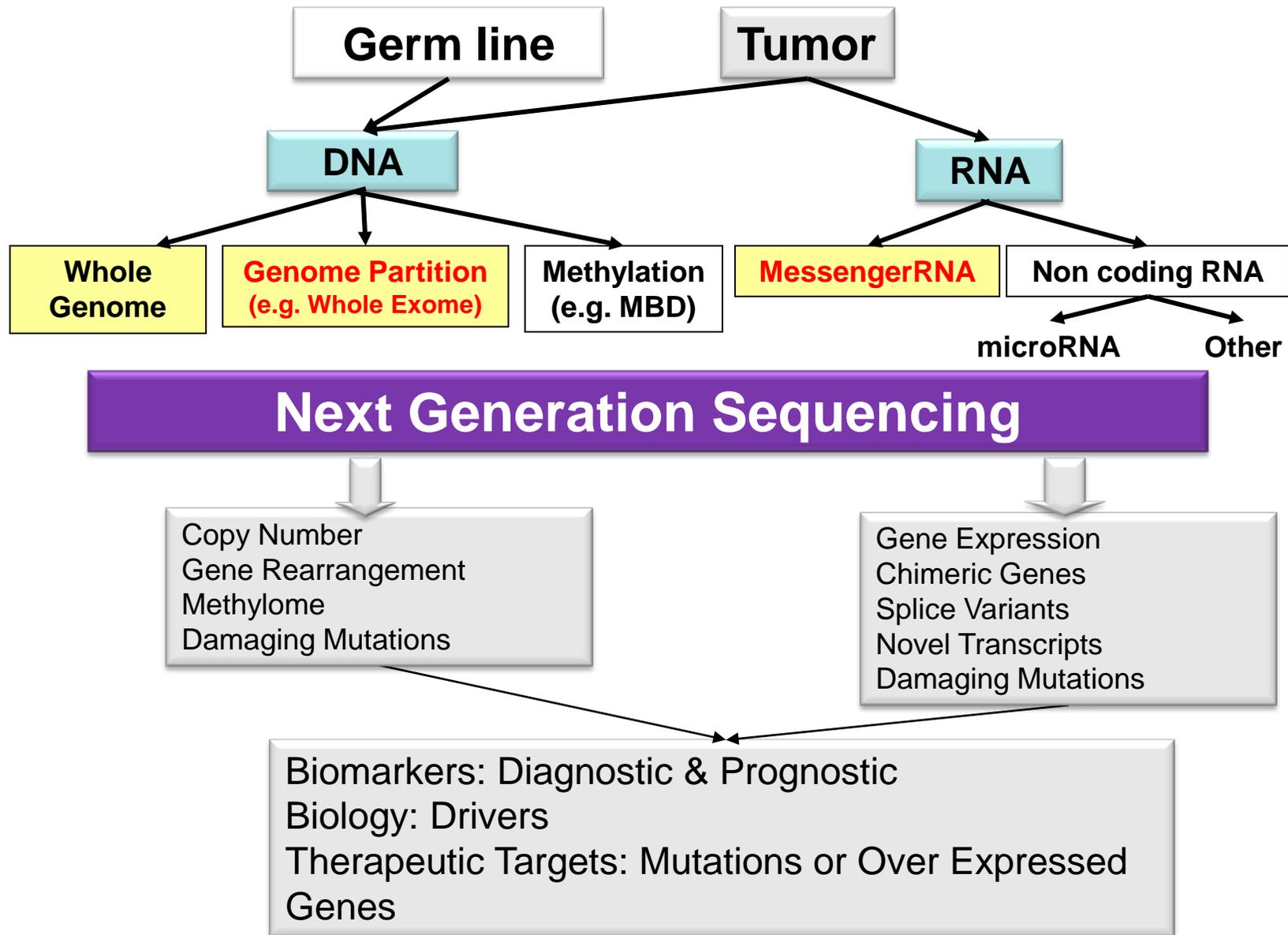


Human Genome Project (1990-2003)
13 years
Pooled DNA
Total US cost ~ \$3,000,000,000

**300 GB/run/week = 2 human
genomes @ 30x coverage**

**Next Generation
Sequencing will become a
routine clinical laboratory
test**

Current and Future Directions: Applying Novel Genomic Approaches for the Comprehensive Analysis of the Pediatric Cancer Genome- Catalog "ALL" the Changes in the Genome



Next Generation Sequencing will Identify Biomarkers and Driver Mutations and Enable Individualized Therapy for Cancer



Roche / 454
Genome Sequencer FLX
Titanium



Illumina / GAI/IIHQ



Life
Technologies
SOLiD v4



Life
Technologies
PI/HiSeq



Life
Technologies
Ion Torrent



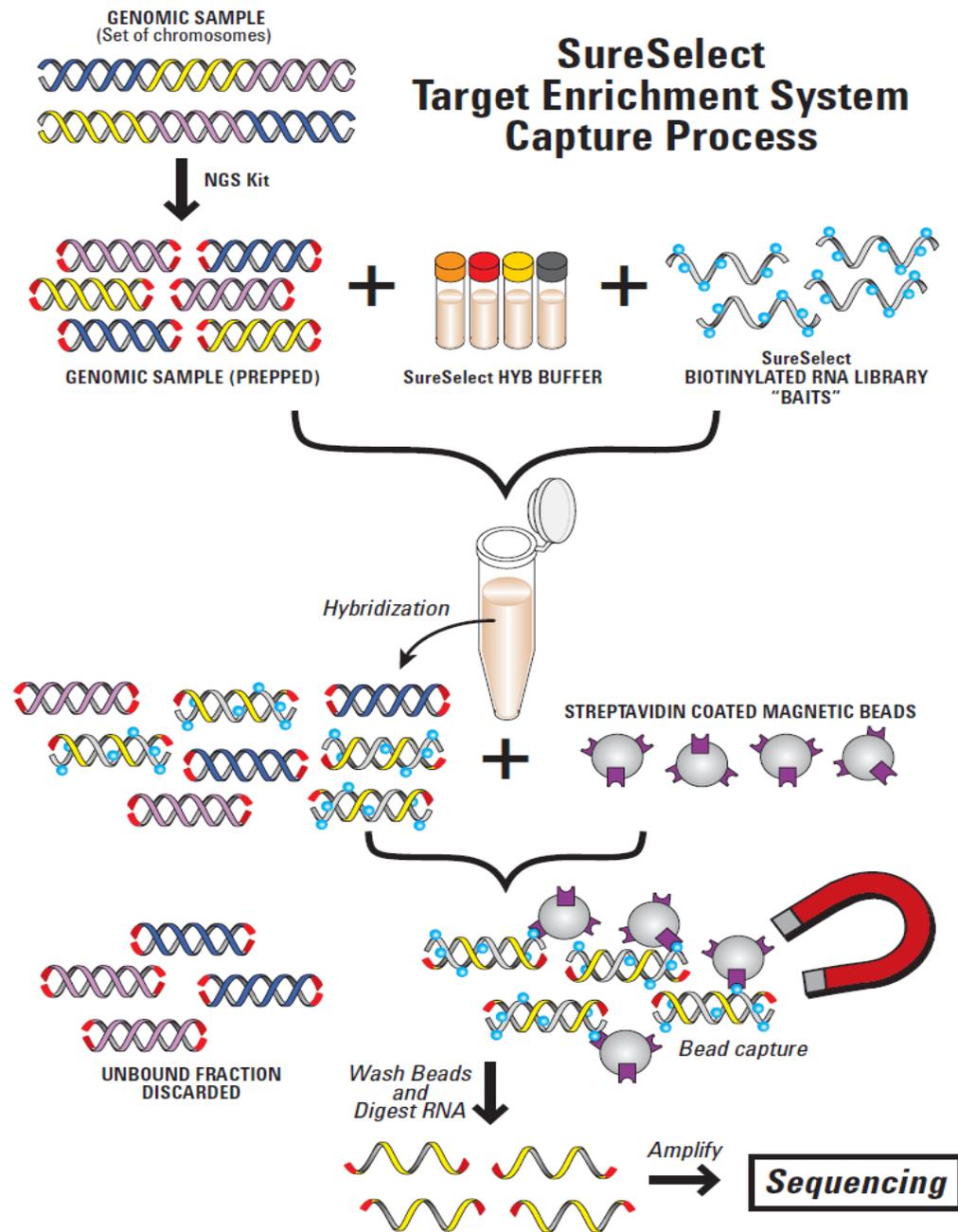
PacBio RS
Ion Torrent



Helicos
HeliScope

Large Scale Pediatric Sequencing Efforts - US

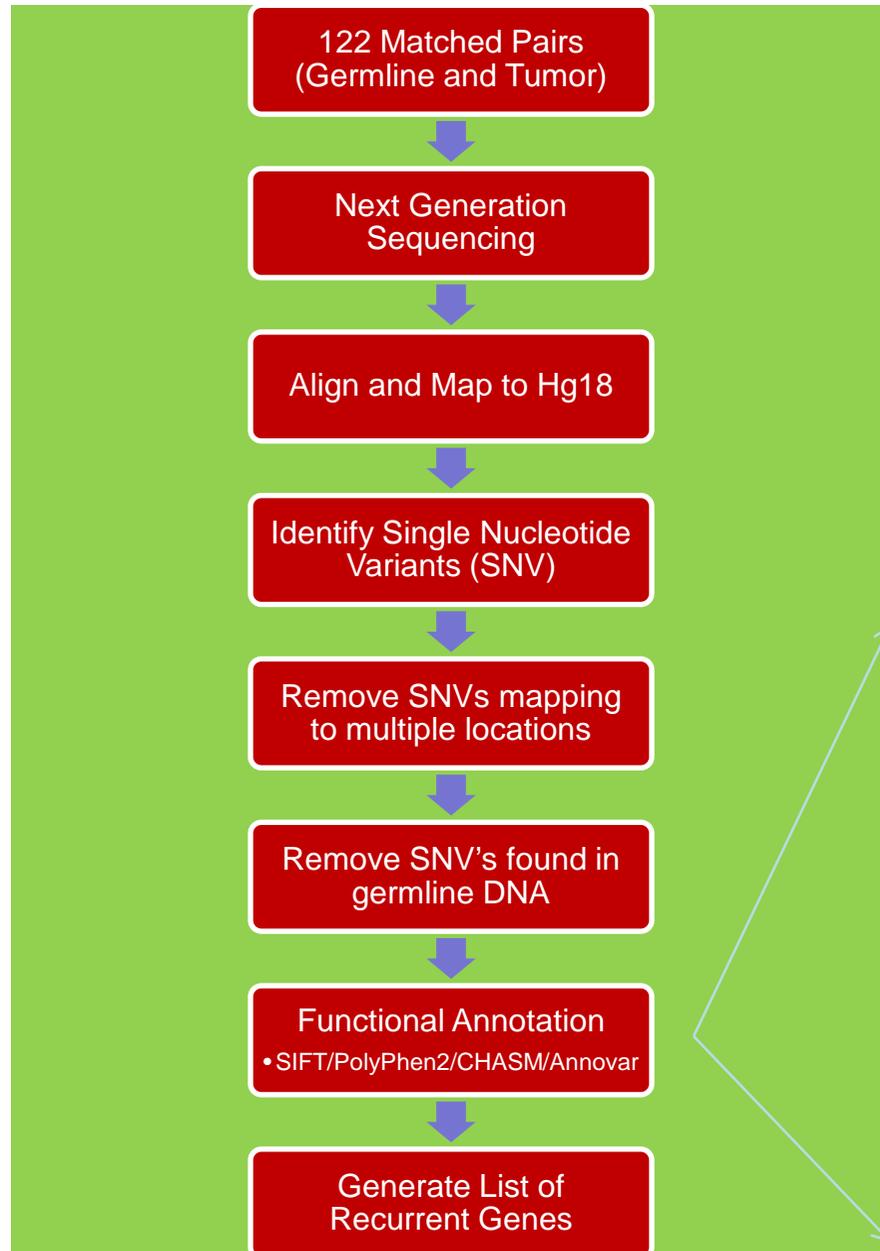
1. Therapeutically Applicable Research to Generate Effective Treatments: TARGET (NCI funded)
 - i. Neuroblastoma (COG, CHOP, CHLA, Broad, BCCA [CCR, NCI](#))
 - ii. ALL
 - iii. Osteosarcoma (COG, BCM, CHLA, [-CCR, NCI](#))
 - iv. AML
 - v. Wilm's Tumor
2. Pediatric Cancer Genome Project (St. Jude and Washington University St. Louis)
 - i. ALL (TARGET)
 - ii. 600 pediatric cancers
3. Center for Cancer Research, NCI
 - i. Oncogenomics Section-Khan- [Collaboration with Life Technologies](#)
 1. Neuroblastoma- (Exome, Transcriptome)- [TARGET](#)
 2. Rhabdomyosarcoma- (Exome, Transcriptome, Whole Genome) - [COG](#)
 3. Ewing's Sarcoma- (Exome & Transcriptome) - [COG](#)
 - ii. Meltzer and Khan
 1. Osteosarcoma- (Exome & Transcriptome)- [COG, BCM, CHLA](#)



Whole Exome Sequencing of RMS

- Targeting of all human exomes –protein coding (38Mb)
- Covers 1.22% of the human genome

Oncogenomics NGS Pipeline



- SIFT – prediction of whether an amino acid substitution affects protein function
- Polyphen2 – prediction of impact of amino acid substitution on structure and function
- CHASM – Prediction of somatic mutations are driver vs passenger mutations
- Annovar – functional annotation of somatic mutations

Using Genomics to Individualize Therapy

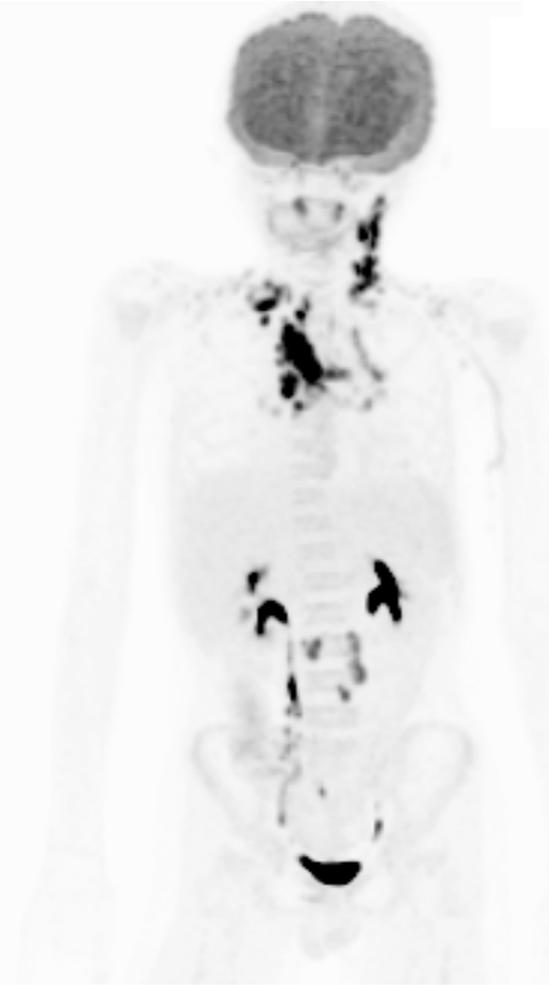
11 year old NPM-ALK positive ALCL, multiply relapsed after combination chemotherapy. Extensive nodal disease. Treated with small molecule ALK-inhibitor (PF-02341066)

Pre-Cycle 1

Post-Cycle 1 (CR)

9/21/2010

10/18/2010



FDG-PET

Courtesy of Brigitte Widemann, MD, Alan S. Wayne, MD (POB)

11-C-0159

**A Feasibility Trial using Molecular-Guided
Therapy for the Treatment of Patients with
Refractory or Recurrent Neuroblastoma**

11-C-0159

A Feasibility Trial using Molecular-Guided Therapy for the Treatment of Patients with Refractory or Recurrent Neuroblastoma Personalized Therapy Protocol

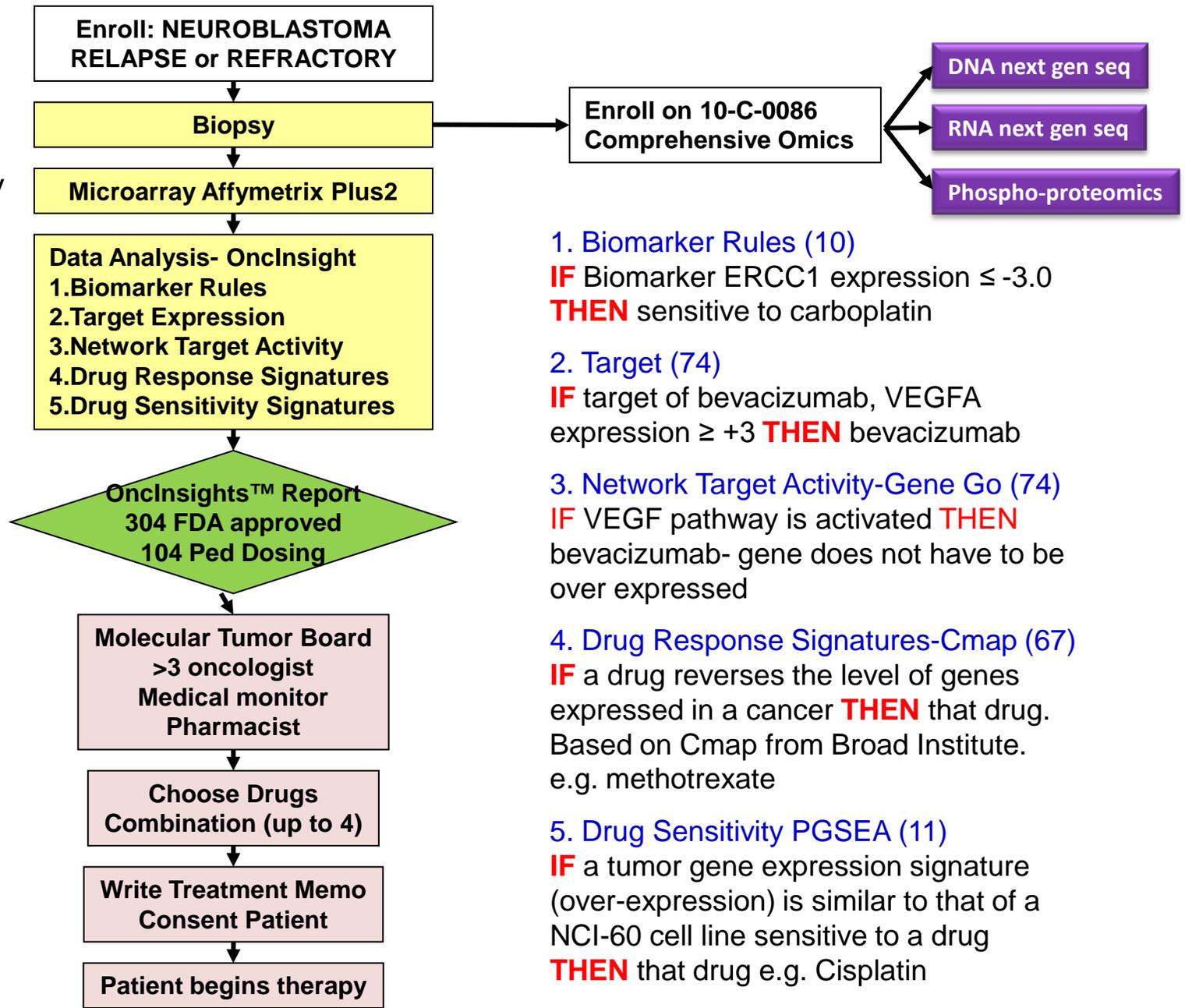
**Neuroblastoma and Medulloblastoma
Translational Research Consortium (NMTRC)
Trial**

- Coordinating Center: Van Andel Research Institute**
- NMTRC Study Chair: Giselle Sholler**
- NCI PI: Javed Khan**
- NCI Lead Associate Investigator: Melinda Merchant**

Schema Molecular Guided Therapy Protocol: 11-C-0159

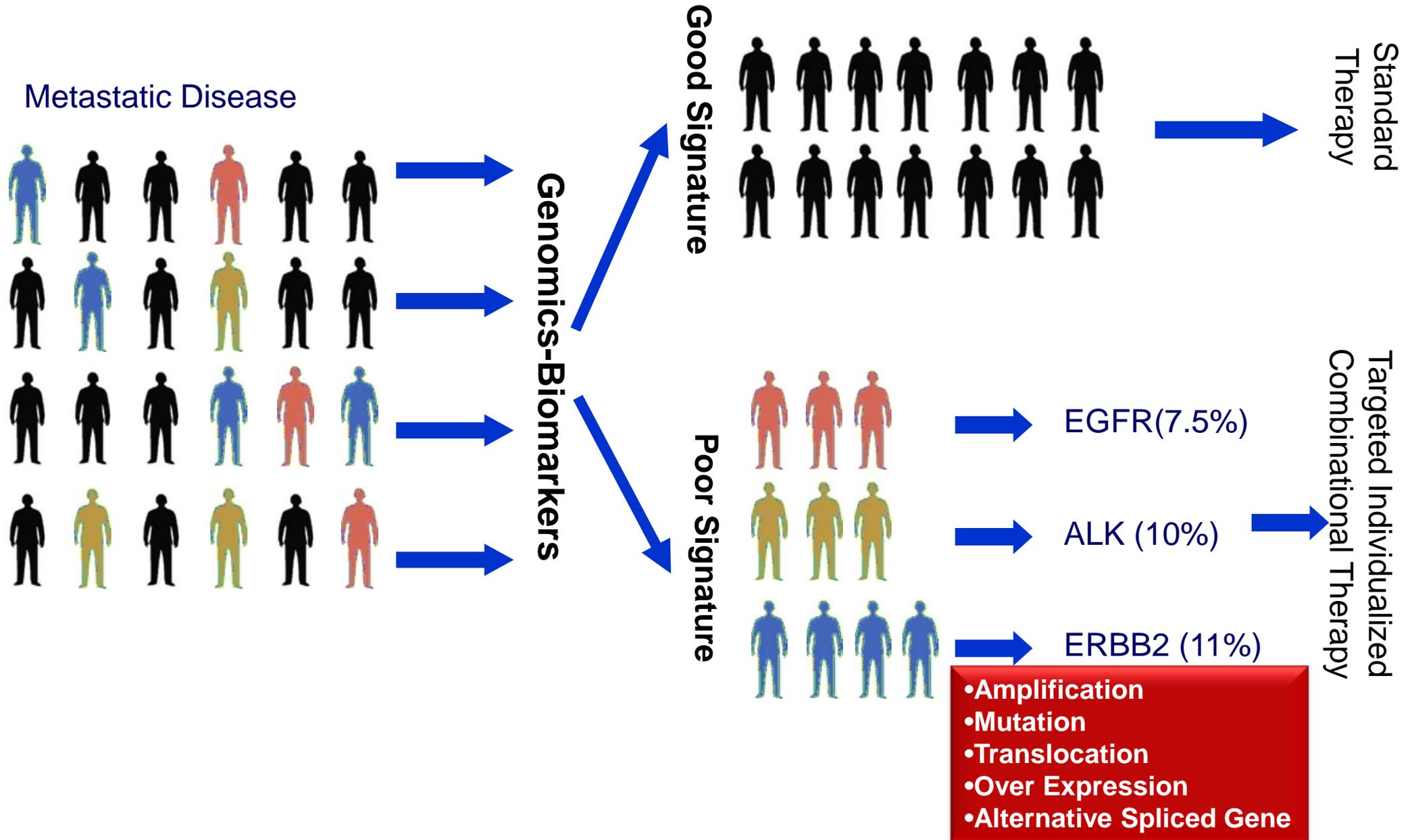
Feasibility
 Enrollment, quality mRNA obtained, gene chip completed, OnInsight report generated, tumor board held, medical monitor review and approval, and completion of 1 cycle of therapy

Biopsy to first dose 21 days (14 days preferred)



1. Biomarker Rules (10)
IF Biomarker ERCC1 expression ≤ -3.0
THEN sensitive to carboplatin
2. Target (74)
IF target of bevacizumab, VEGFA
 expression $\geq +3$ **THEN** bevacizumab
3. Network Target Activity-Gene Go (74)
IF VEGF pathway is activated **THEN**
 bevacizumab- gene does not have to be
 over expressed
4. Drug Response Signatures-Cmap (67)
IF a drug reverses the level of genes
 expressed in a cancer **THEN** that drug.
 Based on Cmap from Broad Institute.
 e.g. methotrexate
5. Drug Sensitivity PGSEA (11)
IF a tumor gene expression signature
 (over-expression) is similar to that of a
 NCI-60 cell line sensitive to a drug
THEN that drug e.g. Cisplatin

Individualized Therapy-The Future



Conclusions and Challenges

Challenges for Individualized Therapy

Technology and Biology

- Microarray fast, next gen slow (1 month) but improving
- Significant false discovery rate for NGS, but improving
- Most of the SNVs are in non protein coding regions (98%)
limitations in knowledge of the biology of these changes
- Protein coding genes: (2%) 25,000-limitations in
knowledge of cancer biology & pathways:- passenger vs.
driver
- Extensive heterogeneity between individuals, within
different tumors of the same individual, and within each
tumor
- >100 germline and >100 somatic non synonymous
mutations. Which to target? Role of germline mutations

Challenges of for Individualized Therapy

Availability of Drugs

- Druggable genome: ~3000
- Genes/proteins with which a drug will interact (DrugBank) ~6,000
- Human genome targets of FDA approved drug ~300 *1
- Tyrosine kinases for which there is a inhibitor in clinical trials ~30 *2
- Tyrosine kinases for which there is a FDA approved inhibitor 18 (~10 drugs) *2

*1 Overington et al. Nature Reviews Drug Discovery, vol 5,2006

*2 Tim Harris, Nature Reviews, Drug Discovery, 9, 2010

Conclusions

1. Genomic analysis of cancer identifies biologically relevant diagnostic, prognostic biomarkers and targets for therapy.
2. These are begin translated to the clinic.
3. Powerful emerging tools of next generation sequencing of tumor and germ line will determine the complete genomic portrait of the majority of cancers at the base pair level within the next 2-5 yrs
4. This will lead to the identification of key drivers and will enable the development of future novel therapies
5. Sequencing becoming cost effective. Rapidly approaching the ~\$1000 genome allowing this to be a part of routine clinical lab test.
6. Individualized “Next Generation Medicine” is rapidly being enabled by genomic technologies.

Acknowledgements

Biologists

- Jun Wei
- Young Song
- Tom Badgett
- Catherine Tolman
- Hongling Liao
- Adam Cheuk
- Patricia Tsang
- Susan Yeh*

Bioinformatics

- Qingrong Chen
- Peter Johansson
- Jianbin He
- Xinyu Wen
- Jianjun Wang

Pediatric Oncology Branch

- Melinda Merchant
- Crystal Mackall
- Carol Thiele
- Clinical Team

SAIC

- Tim Harris
- Bob Stephens

NCI Leadership

- John Niederhuber
- Harold Varmus
- Bob Wiltrout
- Lee Helman

Acknowledgements

NCI

- Yanlin Yu
- Glenn Merlino
- Sven Bilke
- Paul Meltzer
- Joon-Yong Chung
- Stephen M. Hewitt
- Stephen J. Chanock
- Arnulfo Mendoza
- Chand Khanna
- Vu Ngo
- Lou Staudt

NHLBI

- James Taylor VI
- Krupa Desai
- Kushal Shah

CHTN/COG

- Stephen J. Qualman

Children's Hospital at Westmead, Australia

- Daniel Catchpoole

Pediatric Cancer Genomes Consortium

NCI-Oncogenomics

- Javed Khan

NCI-Cancer Genetics

- Paul Meltzer

Children's Oncology Group

- Greg Reaman/Peter Adamson
- Doug Hawkins/Stephen Skapek (RMS Biology)

TARGET

- John Maris (CHOP)
- Bob Seeger (CHLA)
- Ching Lau (TXCC)
- Daniela Gerhardt
- Malcolm Smith

BCGSC

- Marco Marra

Broad Inst.

- Matthew Meyerson