

Development of Targeted Therapies for Paediatric Osteosarcoma

Richard Gorlick, M.D.

Vice Chairman, Pediatrics

Division Chief, Pediatric Hematology-Oncology

The Children's Hospital at Montefiore

Associate Professor of Pediatrics and Molecular Pharmacology

Albert Einstein College of Medicine

Asian Oncology Summit

Bali, Indonesia

April 10th 2010



Targeted Therapies are Effective in Tumor's with "Oncogene Addictions"*

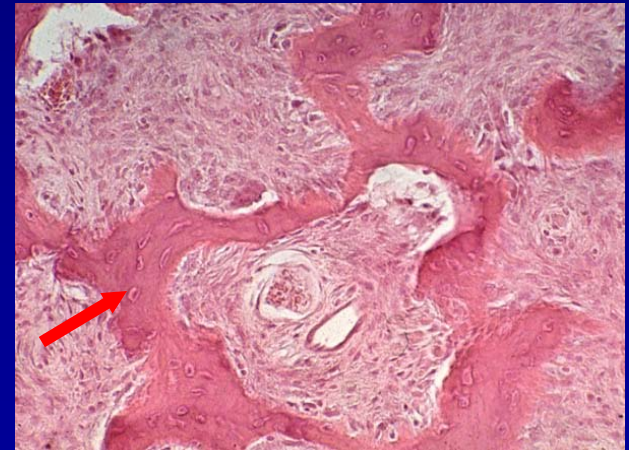
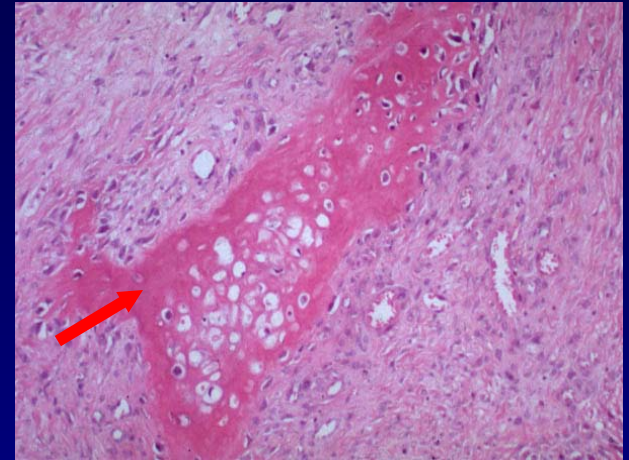
Alteration	Gene	Cancer	Therapy
Amplification	HER-2	Breast	Trastuzumab
Translocation	BCR-ABL	Leukemia	Imatinib
Translocation	PDGF	DFSP	Imatinib
Mutation	c-KIT PDGFR	GIST	Imatinib
Mutation	EGFR	NSCLC	Gefitinib Erlotinib

*Weinstein, Vogelstein, Sawyers, Baselga and numerous others

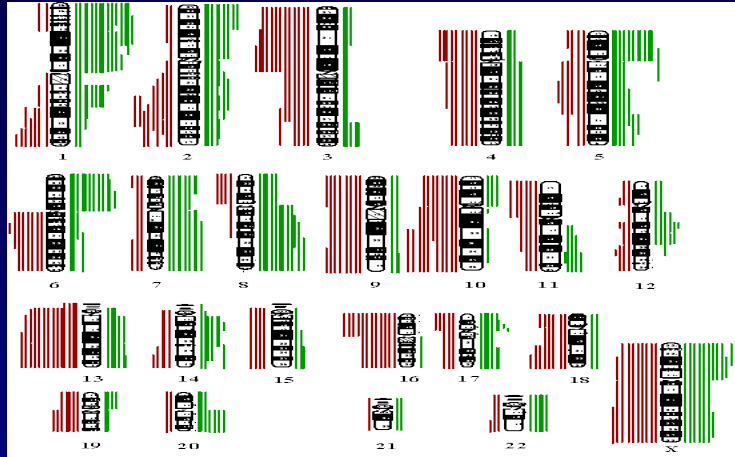


Defining Osteosarcoma

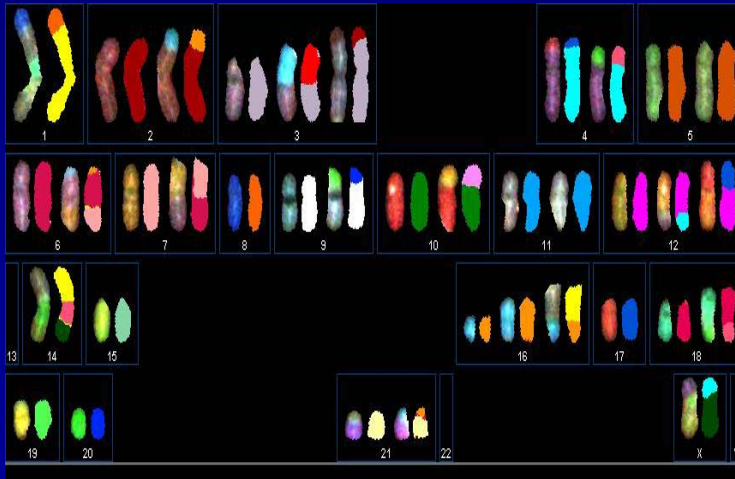
- Osteosarcoma is defined based on a phenotype: a malignant cell which produces osteoid
 - No recurrent genetic alteration – translocation or amplification – is assessed to assist in making the diagnosis.
 - No characteristic protein product – immunohistochemistry - is performed to assist in making the diagnosis.
 - Considerable variability exists in its histologic appearance
- Despite the phenotypic definition the clinical disease is relatively homogeneous.



Osteosarcoma is a Member of the Group of Sarcomas which are Genetically Complex



CGH of 30 OS biopsy samples



Spectral karyotyping of one OS sample

Sarcomas	Non-translocation Associated (2/3 of sarcomas)
Translocations	Nonreciprocal Nonspecific
Karyotypes	Usually Complex
p53 Pathway	Frequently Altered Weakly prognostic if altered
Etiologic Factors	Associated with Li-Fraumeni, hereditary retinoblastoma and/or radiation.



It is difficult to create a complete list of genes that have been studied in osteosarcoma

Cell Cycle Regulation

p53
Rb
MDM2
COPS3
p16^{INK4A}
p14^{ARF}
p21^{waf1}
CDK4
CCND1

Regions of Amplification

CDC5L
PMP22

Oncogenes

Fos
MYC

Paget's

SQSTM1

Signaling

EGF
HER2
ErbB-4
PTEN
VEGF
PDGF
IGF/IGFR
MET/HGF
MAPK7/8

Src
PI3K/AKT
mTOR

Metastases

Ezrin
CXCR4
SDF1

Differentiation

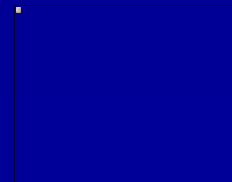
Wnt
 β -catenin
LRP5
TGF- β
RANK
PTH/PTHrP
Runx2
Cbfa1
BMP/BMPR

DNA

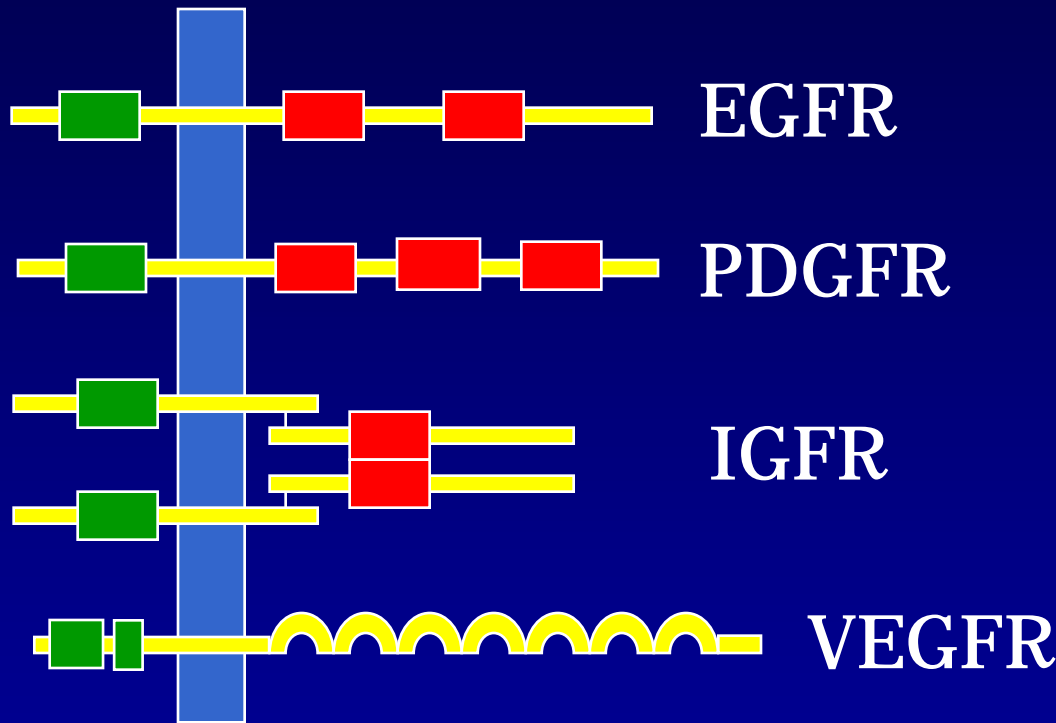
RECQ helicases
hTERT/ALT

Drug Resistance

MDR1
RFC
DHFR



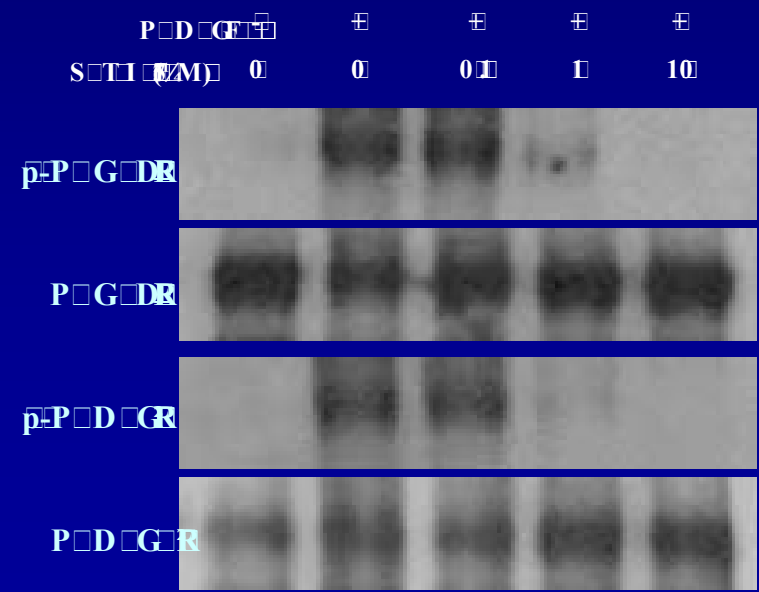
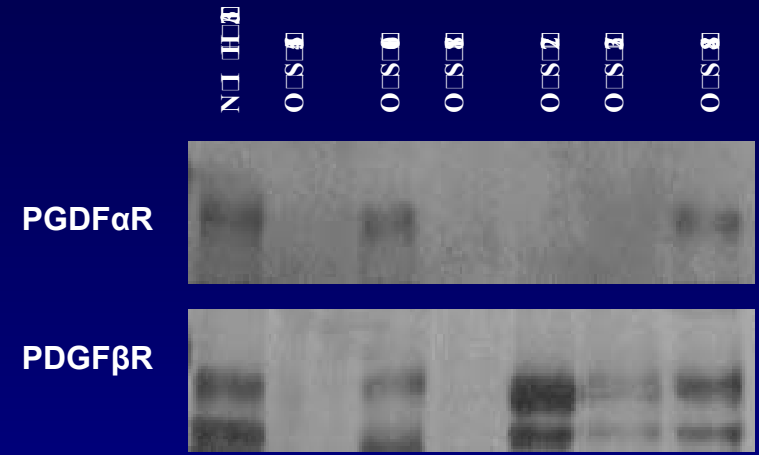
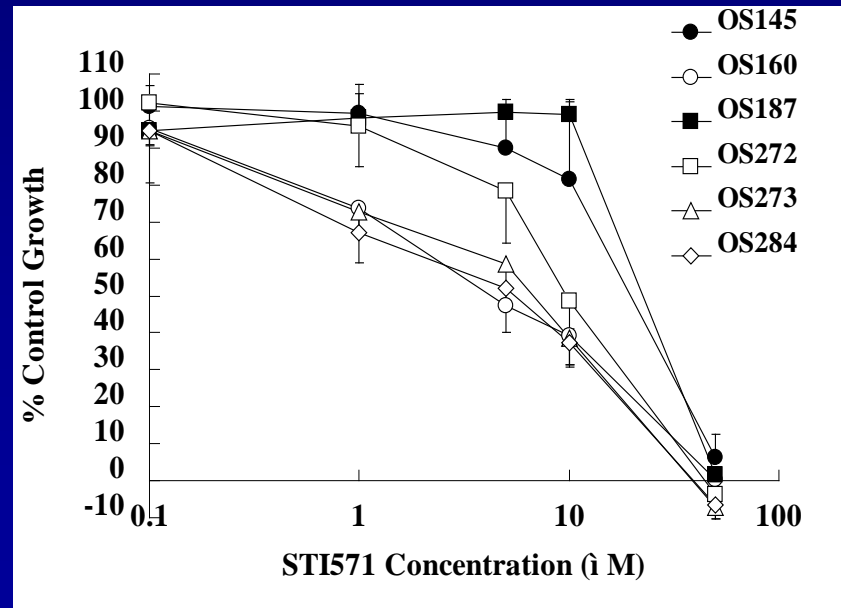
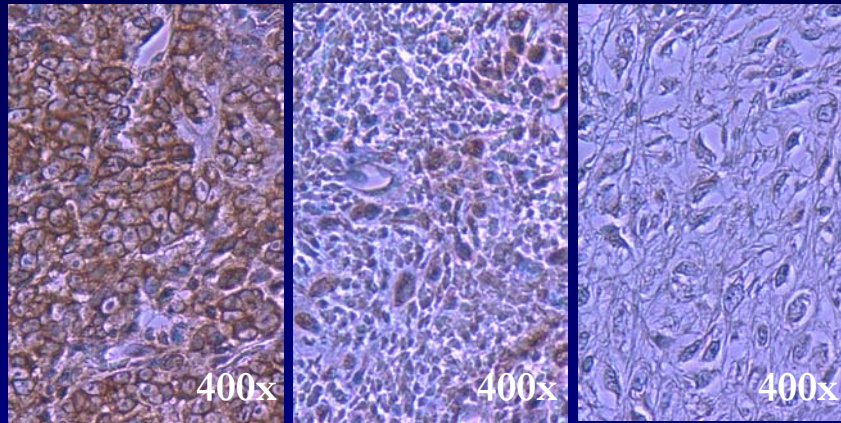
Osteosarcoma has numerous redundant genetic alterations all of which are not necessary to produce its phenotype



Tumors are defined by their unregulated proliferation and loss of contact inhibition. Osteosarcoma expresses numerous growth factor receptors on its surface all of which can in theory drive proliferation

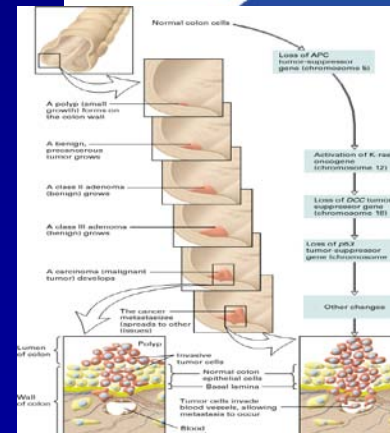
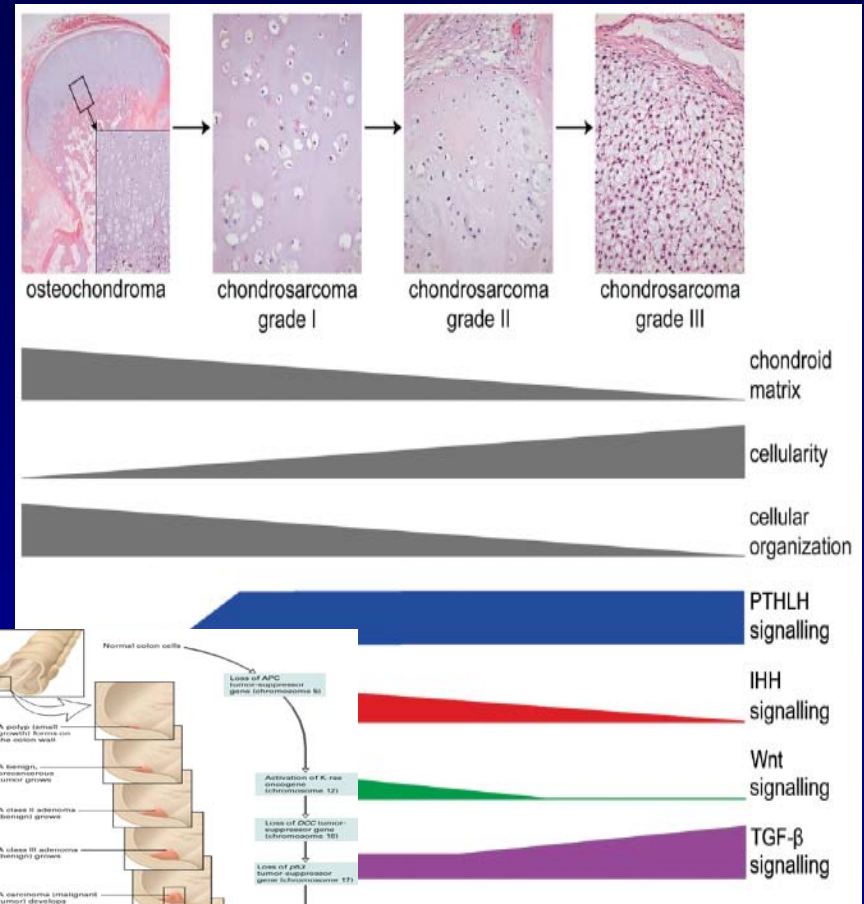
The presence of a growth factor receptor does not prove it is involved in osteosarcoma pathogenesis or its necessity for tumor maintenance

PDGFR is expressed in osteosarcoma but it is not sensitive to Imatinib Mesylate despite inhibition of PDGFR phosphorylation suggesting this pathway is not central in its pathogenesis



Defining the Pathogenesis of Osteosarcoma

- Many malignancies which afflict older individuals are genetically complex but many of these have pre-malignant lesions which can help understand the genetic steps underlying tumor formation and progression.
- Pediatric malignancies generally do not have defined precursor lesions and in some cases a defined cell of origin.
- It is difficult to define the pathogenesis of tumors that are both genetically complex and lack precursor lesions.



L.Hameetman,¹ L.B. Rozeman,¹ M. Lombaerts,¹ J. Oosting,¹ A.H.M. Tamminga,² A.M. Cleton-Jansen,¹ J.M.G. Bovee¹ and P.C.W. Hogendoom^{1*}

Journal of Pathology
J Pathol 2006; 209: 501–511

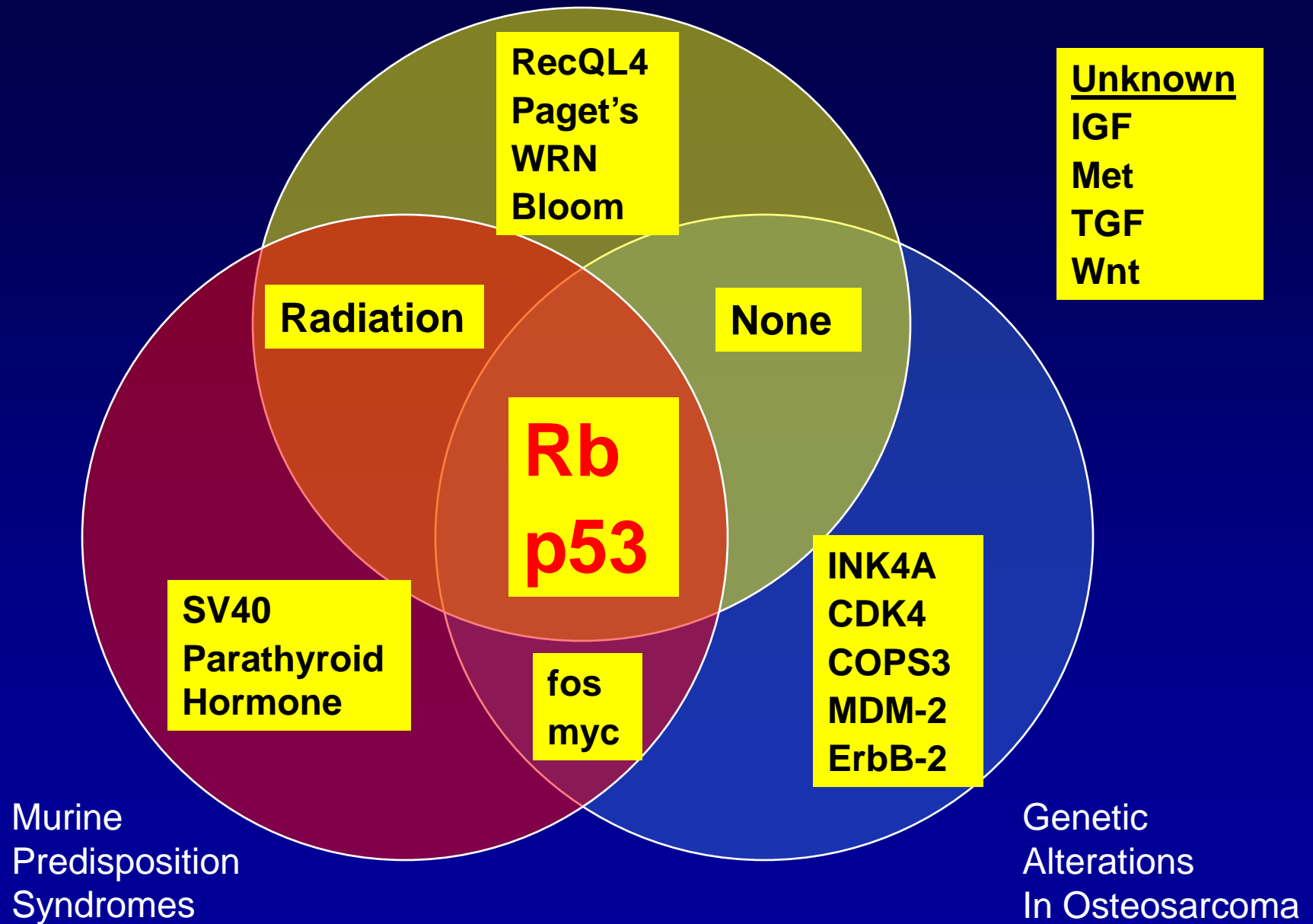
Lodish, et al

Many Clues Identify Factors that may be Associated with Osteosarcoma Development

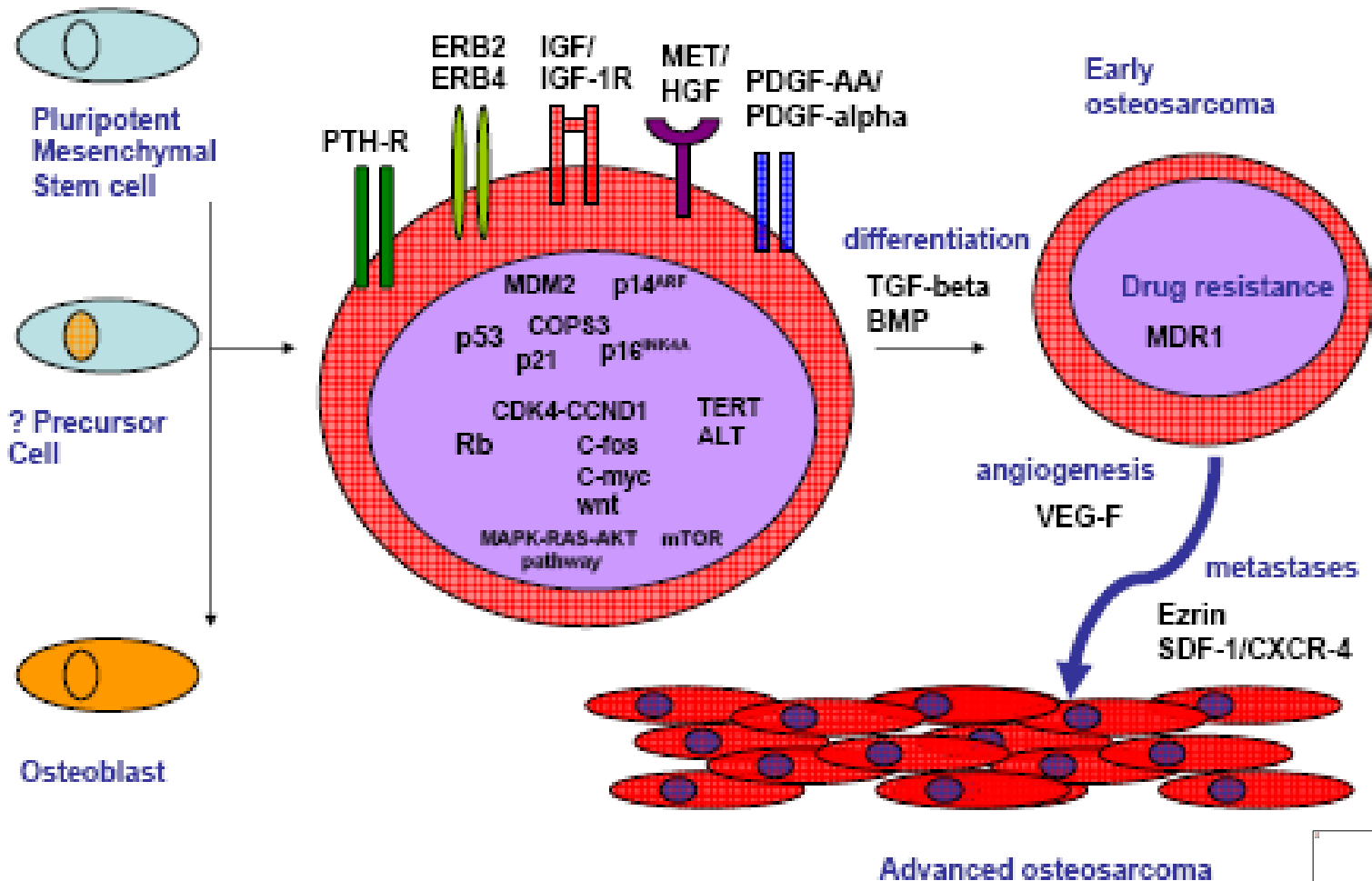
- Murine Predisposition Models
 - p53 knock out mouse
 - SV40 Tag transgenics
 - Myc Transgenics
 - Fos transgenics/Parathyroid hormone
- Human Predisposition Models
 - Hereditary Retinoblastoma (Rb)
 - Li-Fraumeni Syndrome (p53)
 - Rothmund-Thomson (RecQL4)
 - Werner Syndrome (WRN)
- Epidemiology
 - Radiation
 - Bone turnover – Paget's Disease
 - Growth



Human Predisposition Syndromes



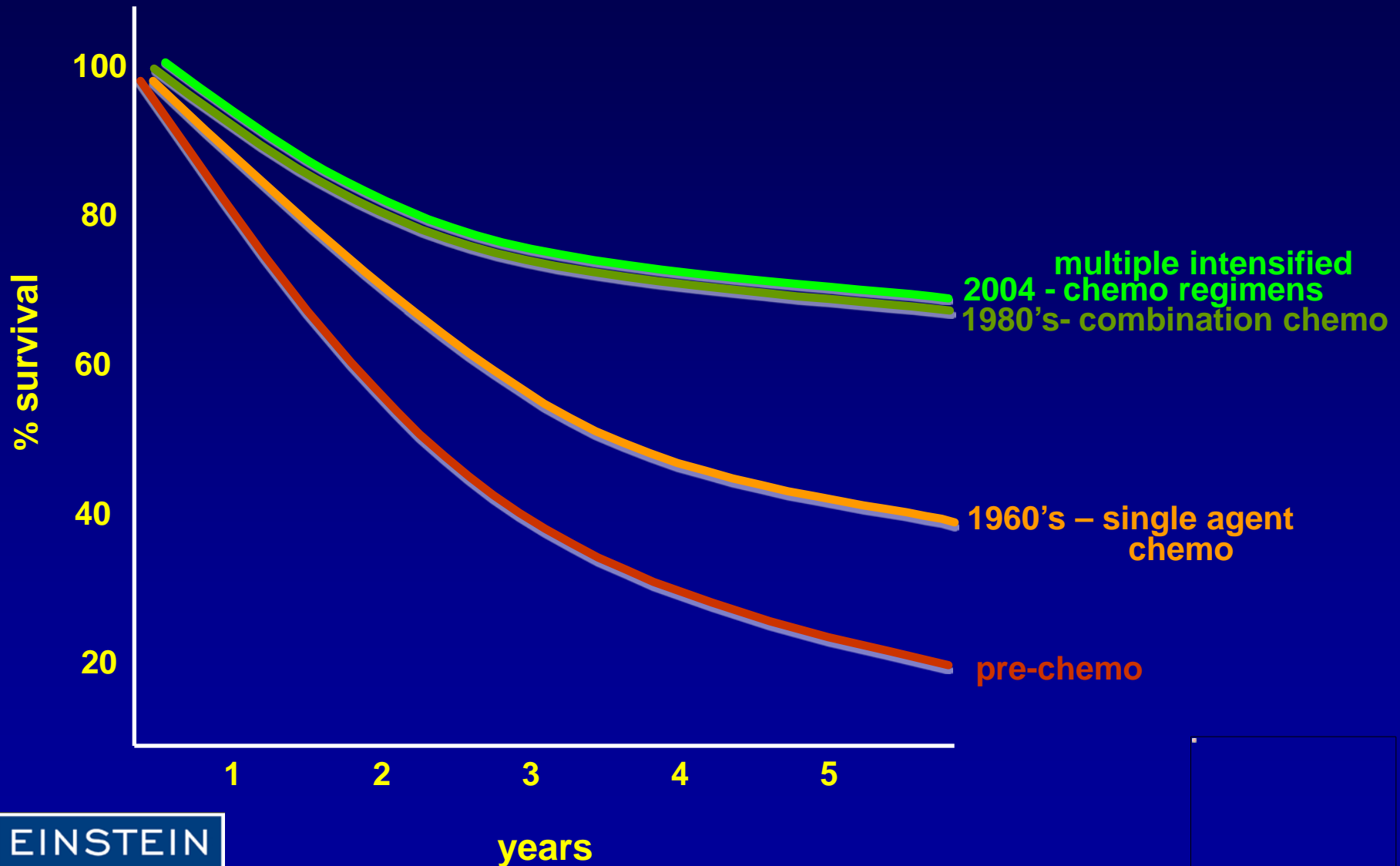
Current models include a large number of factors as potentially involved in osteosarcoma pathogenesis



Despite having a great deal of data characterizing osteosarcoma we have little molecular understanding of its nature because of its genetic complexity, redundancy and our inability to simplify the system.



SURVIVAL OF PATIENTS WITH LOCALIZED OSTEOSARCOMA



multiple intensified
2004 - chemo regimens
1980's - combination chemo

1960's - single agent
chemo

pre-chemo



Clinical Goals of Sarcoma Research

- Biology studies may identify prognostic factors which may serve as a basis for stratification of therapy.
 - In the majority of pediatric malignancies such as ALL, therapy stratified by risk is the norm and is believed to be partly responsible for the favorable outcomes.
- Biology studies may help to prioritize clinical trials of new therapeutic agents.
 - Sarcomas are rare malignancies. Empiric testing of all new agents in these diseases is NOT feasible.



Prior Studies of Prognostic Factors have led to Subsequent Contradictory Reports – p-glycoprotein or HER2

EXPRESSION OF P-GLYCOPROTEIN IN HIGH-GRADE OSTEOSARCOMAS IN RELATION TO CLINICAL OUTCOME

NICOLA BALDINI, M.D., KATIA SCOTLANDI, M.S., GIOVANNI BARBANTI-BRODANO, M.D., MARIA CRISTINA MANARA, M.D., DANIELA MAURICI, M.S., GAETANO BACCI, M.D., FRANCO BERTONI, M.D., PIERO PICCI, M.D., SANDRA SOTTILI, M.S., MARIO CAMPANACCI, M.D., AND MASSIMO SERRA, M.S.

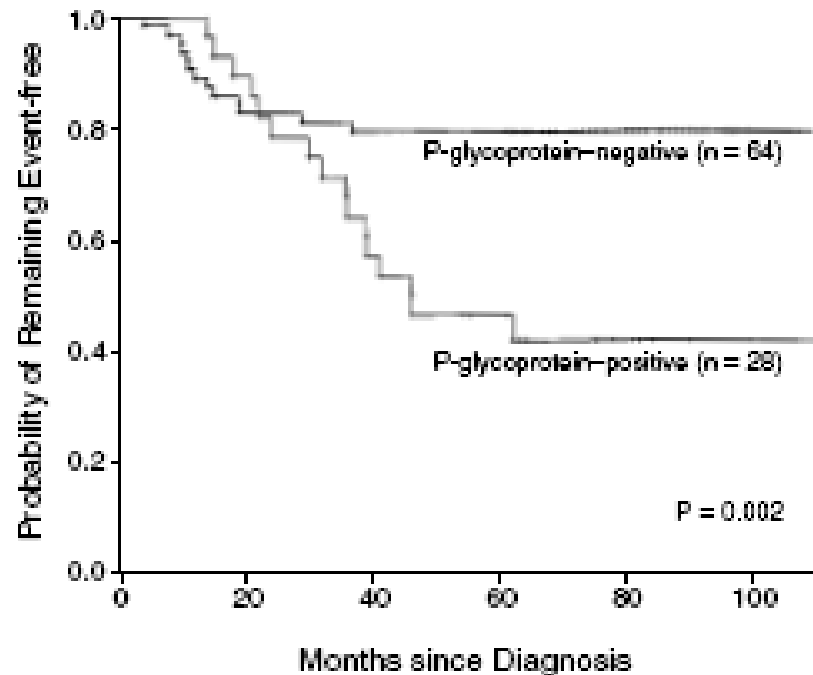


Figure 2. Probability of Event-free Survival in 92 Patients with Osteosarcoma and either P-Glycoprotein-Positive or P-Glycoprotein-Negative Tumors.

Multiple Drug Resistance in Osteogenic Sarcoma: INT0133 From the Children's Oncology Group

Clady L. Schwarz, Richard Gorlick, Lisa Teo, Mark Krailo, Zhongli Chen, Allen Gorrie, Holcombe E. Grier, Mark L. Bernstein, and Paul Meyers

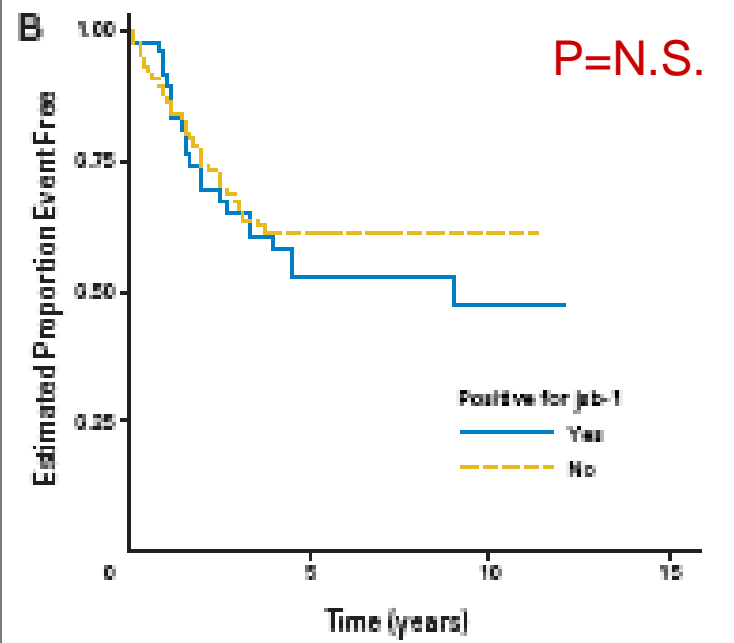
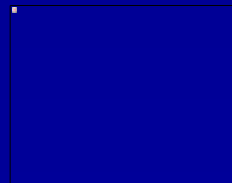


Fig 3. (A) Event-free survival (EFS) for C-424-positive versus -negative non-metastatic patients at study enrollment. (B) EFS for J55-1-positive versus -negative nonmetastatic patients at study enrollment.

Potential reasons for failure to validate prognostic factors

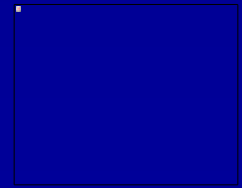
- Single institution retrospective studies have not identified true prognostic factors.
 - Limited Power
 - Insufficiently robust – inadequate predictive value
- The prognostic factor or the method for assessing it is insufficiently robust to be relevant to all osteosarcoma patient cohort.
 - Different homogeneous patient populations
- Differences in tissue handling or processing in multi-institutional studies and changes in method and/or interpretation have led to the inability to reproduce the results.
- Other
 - A factor must be measured in a sufficiently standardized manner and remain prognostic across a prospective multi-institutional study in order to be clinically relevant. Only factors identified as part of large multi-institutional biology studies are likely to meet these criteria.



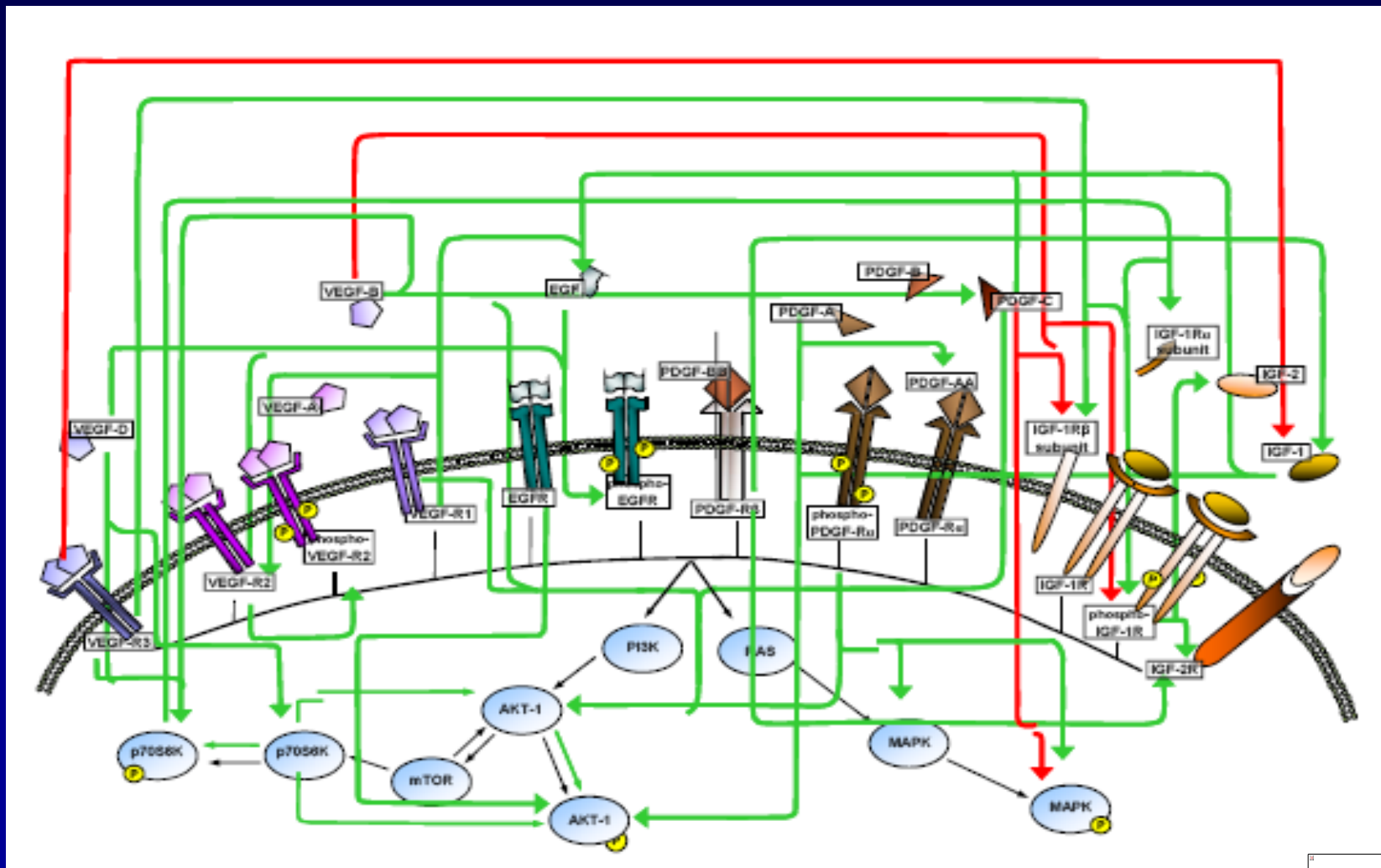
Children's Oncology Group's OS Biology Study

- Biology study development initiated in 1998
- All OS patients aged ≤ 40 years were eligible for participation
- Specimen collection was centralized and predominantly performed through the Cooperative Human Tissue Network
- Access to tissue was through an application process and was open to all investigators

Opened to accrual	9/15/1999
Total accrual	952
Accrual rate on study	100/yr
CHTN-banked OS	
Frozen tumor (snap or embedded in OCT)	557
WBCs (from blood or bone marrow)	952
Serum	899 cases
Paraffin tumor blocks	234 cases
Unstained/stained slides	601 cases
16 different investigators have been provided OS tissue.	



Assessments of Pathways Rather than One Gene or Protein at a Time may be more Informative



New Technologies Offer Considerable Promise for Understanding Complex Malignancies

Somatic mutations affect key pathways in lung adenocarcinoma

Li Ding^{1*}, Gad Getz^{2*}, David A. Wheeler^{3*}, Elaine R. Mardis¹, Michael D. McLellan¹, Kristian Cibulskis², Carrie Sougnez², Heidi Greulich^{2,4}, Donna M. Muzny⁵, Margaret B. Morgan³, Lucinda Fulton¹, Robert S. Fulton¹, Qunyan Zhang⁵, Michael C. Wendl¹, Michael S. Lawrence², David E. Larson¹, Ken Chen¹, David J. Dooling¹, Aniko Sabo³, Alicia C. Hawes³, Hua Shen³, Shalini N. Jhangiani³, Lora R. Lewis³, Otis HalP, Yiming Zhu³, Tittu Mathew³, Yanru Ren³, Jiqiang Yao³, Steven E. Scherer³, Kerstin Clerc³, Ginger A. MetcalF, Brian Ng³, Aleksandar Milosavljevic³, Manuel L. Gonzalez-Garay³, John R. Osborne¹, Rick Meyer¹, Xiaoj Shi¹, Yuzhu Tang¹, Daniel C. Koboldt¹, Ling Lin¹, Rachel Abbott¹, Tracie L. Miner¹, Craig Pohl¹, Ginger Fewell¹, Carrie Haipek¹, Heather Schmidt¹, Brian H. Dunford-Shore¹, Aldi Kraja⁵, Seth D. Crosby¹, Christopher S. Sawyer¹, Tammi Vickery¹, Sacha Sander¹, Jody Robinson¹, Wendy Winckler^{2,4}, Jennifer Baldwin², Lucian R. Chiriac^{6,7}, Amit Dutt^{2,4}, Tim Fennell², Megan Hanna^{2,4}, Bruce E. Johnson⁴, Robert C. Onofrio², Roman K. Thomas^{8,9}, Giovanni Tonon⁴, Barbara A. Weir^{2,4}, Xiaojun Zhao^{2,4}, Liuda Ziaugra², Michael C. Zody², Thomas Giordano¹⁰, Mark B. Orringer¹¹, Jack A. Roth¹², Margaret R. Spitz¹³, Ignacio I. Wistuba^{12,14}, Bradley Ozenberger¹⁵, Peter J. Good¹⁵, Andrew C. Chang¹¹, David G. Beer¹¹, Mark A. Watson¹⁶, Marc Ladanyi^{17,18}, Stephen Broderick¹⁷, Akihiko Yoshizawa¹⁷, William D. Travis¹⁷, William Pao^{17,18}, Michael A. Province⁵, George M. Weinstock¹, Harold E. Varmus¹⁹, Stacey B. Gabriel², Eric S. Lander², Richard A. Gibbs², Matthew Meyerson^{2,4} & Richard K. Wilson¹

Vol 455 | 23 October 2008 | doi:10.1038/nature07423

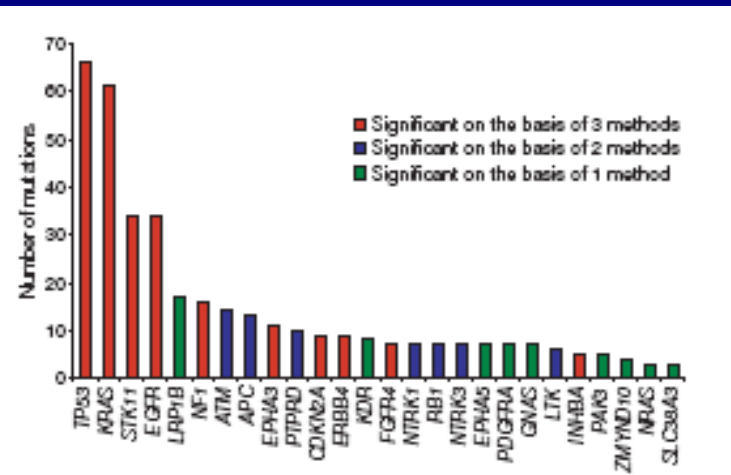
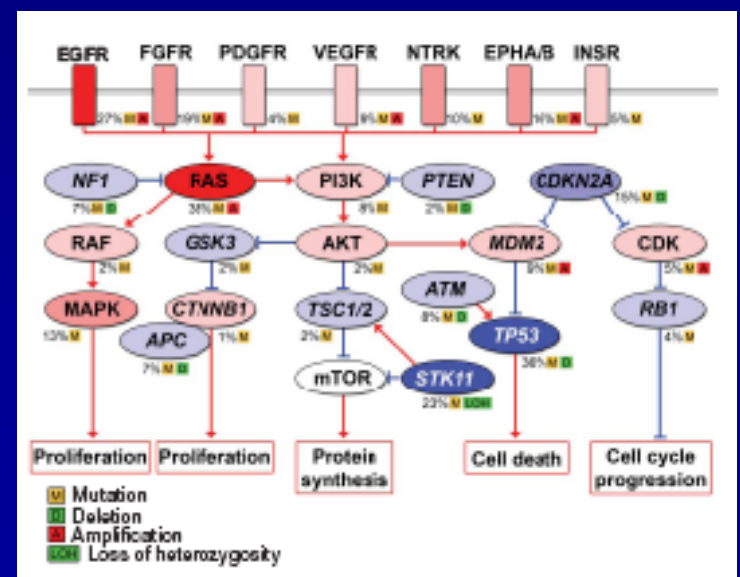


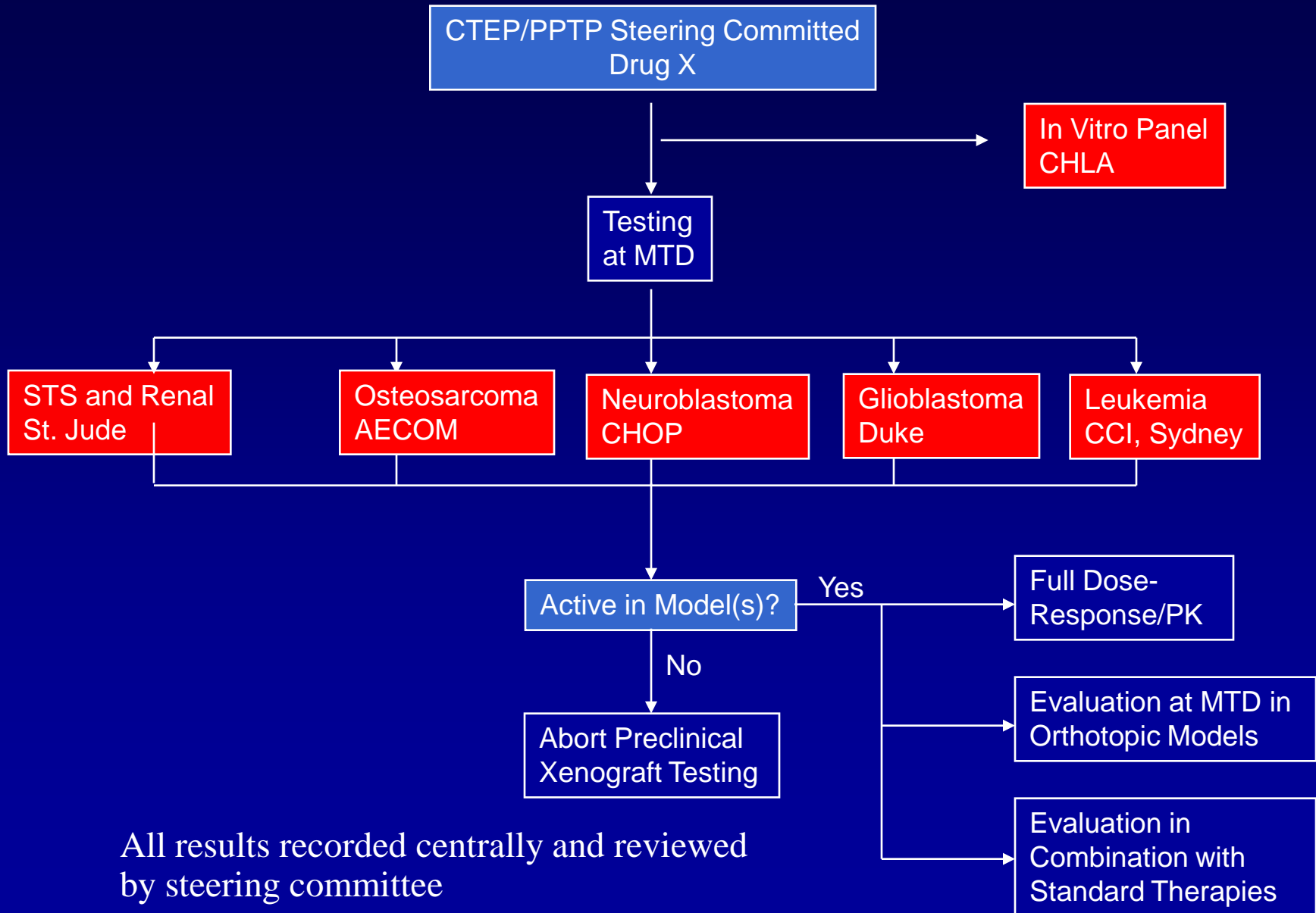
Figure 1 | Significantly mutated genes in lung adenocarcinomas. The height of the bars represents the number of somatic mutations in each



Increasing Focus on Pre-clinical Identification of Effective Targeted Therapy

- If a drug targets a pathway which is central to a tumor's viability "gene addicted" we would expect it to be efficacious.
- As long as normal cells were less dependent upon the pathway a therapeutic index should exist.
- Although few drugs are developed specifically for sarcomas, pathways central to some sarcomas may overlap with those of more common cancers.
- Numerous new drugs exist and there are too few osteosarcoma patients to test them all clinically.

Pediatric Preclinical Testing Program



All results recorded centrally and reviewed by steering committee

PPTP Completed Experiments

Evaluated:

Proteasome inhibitor (bortezomib)

HSP90 inhibitor (17-DMAG)

Kinesin spindle protein inhibitor (Ispinesib)

VEGF receptor antagonist (AZD-2171)

SRC/Tyrosine Kinase inhibitor (Dasatinib)

ERB1/ERB2 inhibitor (Lapatinib)

ERB2 Inhibitor (ABT-263)

mTor Inhibitor (rapamycin)

Multitargeted Tyrosine Kinase Inhibitor (Sunitinib, Sorafenib)

IGF-1 Receptor monoclonal antibody (19D12, IMC-A12, R1507)

Hypoxia Sensitizing Mustard (PR-104)

Polyamine Analogue (PG11047)

AKT Inhibitor (GSK909693)

MEK Inhibitor (AZD6244)

Aurora Kinase Inhibitor (MLN8237)

Bcl-2 Inhibitor (ABT-263)

Standards:

Cyclophosphamide

Vincristine

Cisplatin

Topotecan

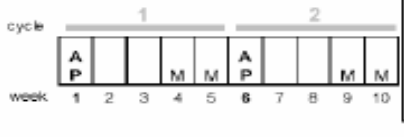


- Demonstrated activity in osteosarcoma xenografts



The Current North American National Cooperative Group Osteosarcoma Study is an International Study

A - Doxorubicin 37.5mg/m²/d d1, d2
 P - Cisplatin 60mg/m²/d d1, d2
 M - Methotrexate 12g/m²
 E - Etoposide 100mg/m²/d
 I - Ifosfamide 2.8g/m²/d
 i - Ifosfamide 1.8g/m²/d } d1 - d5
 Ifn - Interferon-α 2b 1μg/kg weekly

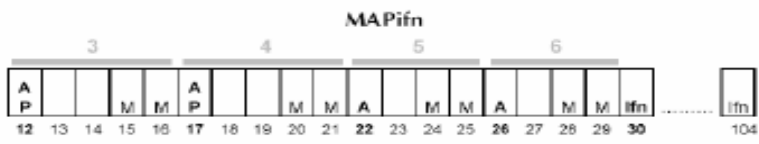


SURGERY

Evaluation of histological response

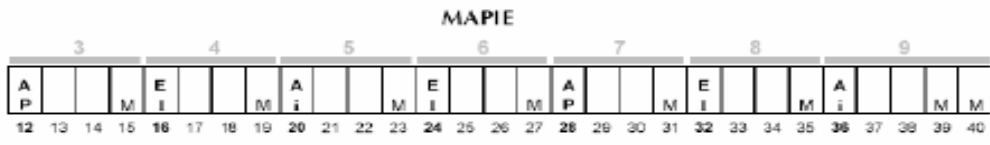
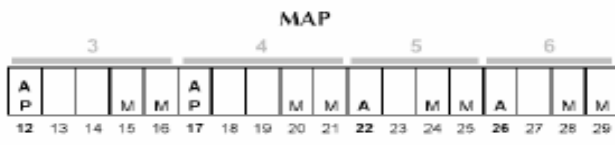
GOOD RESPONSE

RANDOMISE



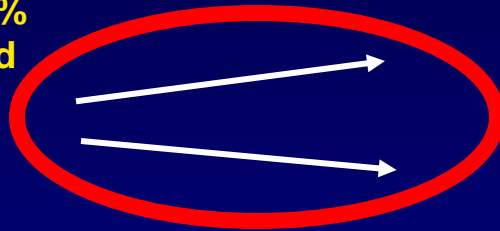
POOR RESPONSE

RANDOMISE

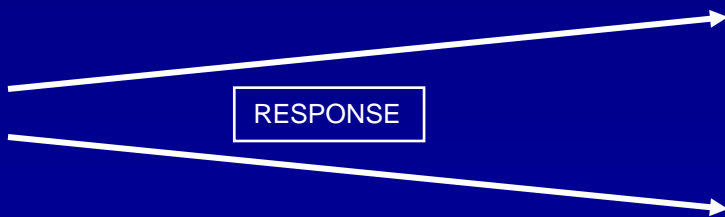
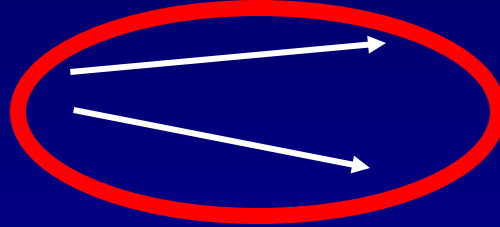


One of the driving forces for performing a European/US study was the required number of patients

In a group of patients with an expected prognosis of 80-95% what is the willingness to add cytotoxic chemotherapy



For the poorer prognosis group want an intervention which has a high perceived likelihood of potentially changing outcome



Statistical Considerations

- Randomize separately for favorable and standard necrosis
 - Need to enroll 1400 patients
- Randomize all patients at enrollment between two regimens.
 - Need to enroll 785 patients



Available IGF-1R Antibodies

Monoclonal Antibodies	Phase 2	Consortium	ES	OS
IMC-A12 (Imclone)	CRC, H&N, Prostate, Pancreatic, sarcoma	COG	Y	Y
AMG 479 (Amgen)	Breast, Pancreatic, EWS		Y	N
R1507 (Roche)	Sarcoma	SARC	Y	Y
CP751,871 (Pfizer)	Breast, CRC, Lung (Ph 3), Prostate, Sarcoma		Y	Y
SCH717454 (Schering- Plough)	CRC, Sarcoma	PPTP	Y	Y
h7c10 (Merck)	?		?	?

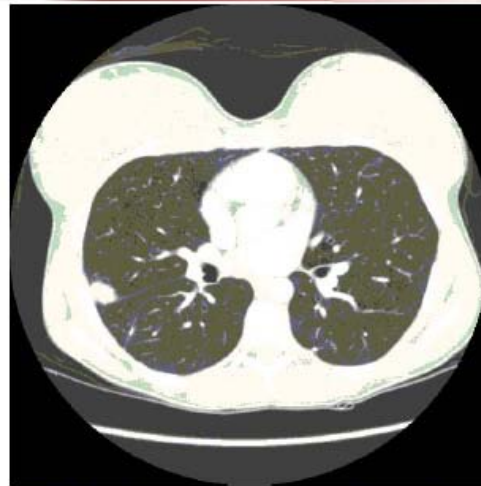
The clinical activity of IGF-1R Ab in Ewing sarcoma is also driving interest

Multiply relapsed Ewing's Sarcoma

Status post standard five drug therapy, salvage therapies



Prior to anti-IGF1R moAb



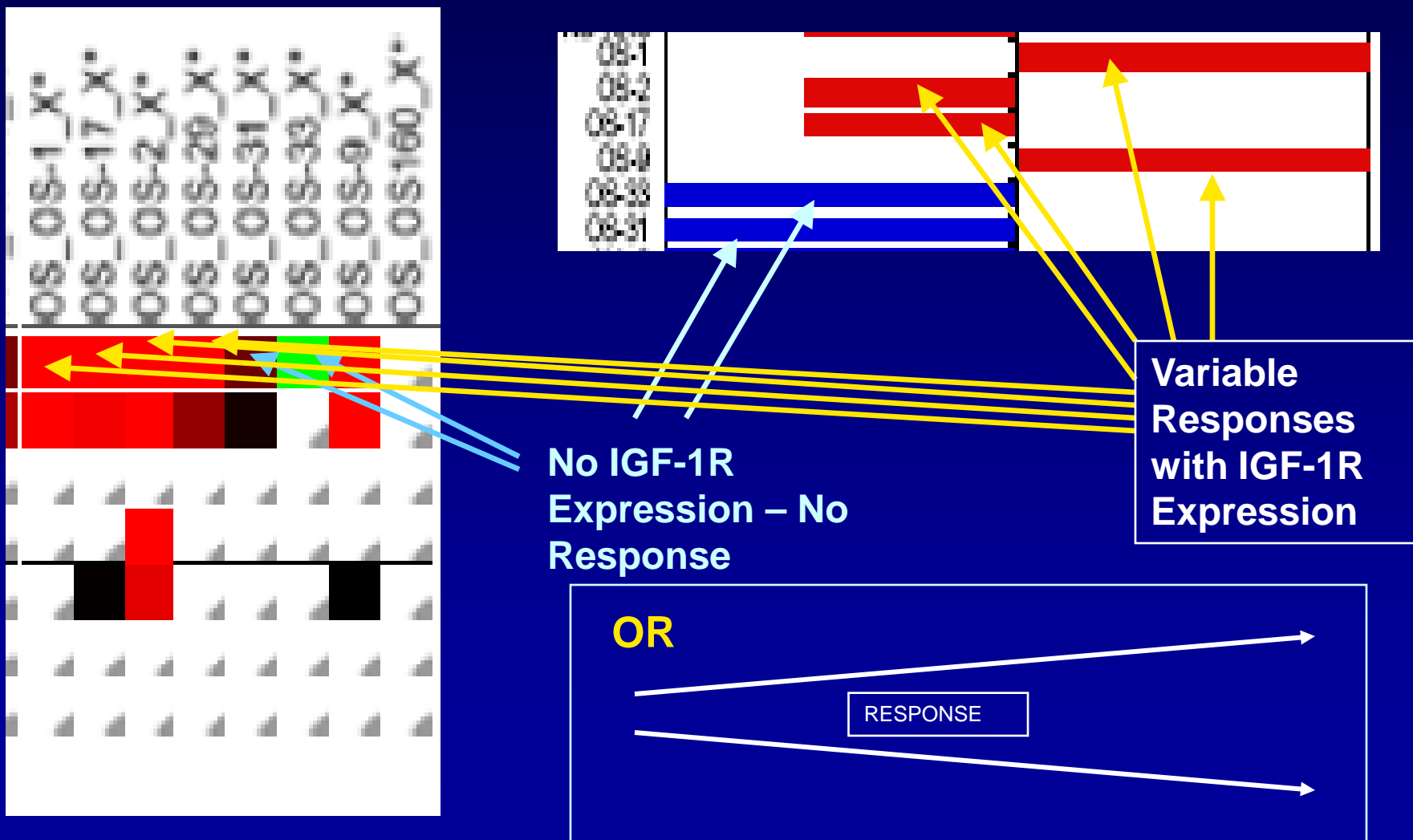
Following one month of weekly dosing of anti-IGF1R moAb

Courtesy of Larry Baker, Univ. of Michigan

SARC Scientific Alliance
for Research
through Collaboration
Optimizing Care

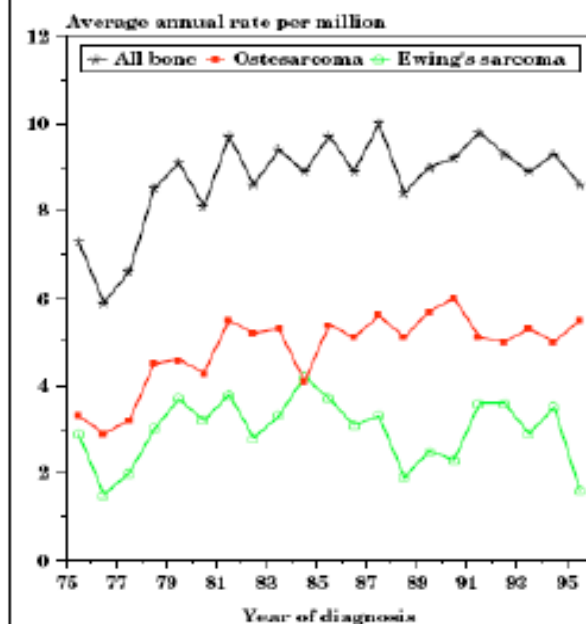


Should IGF-1R Ab ONLY be given to patients whose tumors express IGF-1R? (or study powered for this patient cohort)



Number of Patients per Year with ESFT and osteosarcoma

Figure VIII.10: Trends in bone cancer age-adjusted* incidence rates by histology, age <20 all races both sexes, SEER, 1975-95



*Adjusted to the 1970 US standard population

In the US, 650-700 children and adolescents younger than 20 years of age are diagnosed with bone tumors each year of which approximately 400 are osteosarcoma and 200 are Ewing's sarcoma.

SEER Data

215 Cases/yr

700 Cases/yr

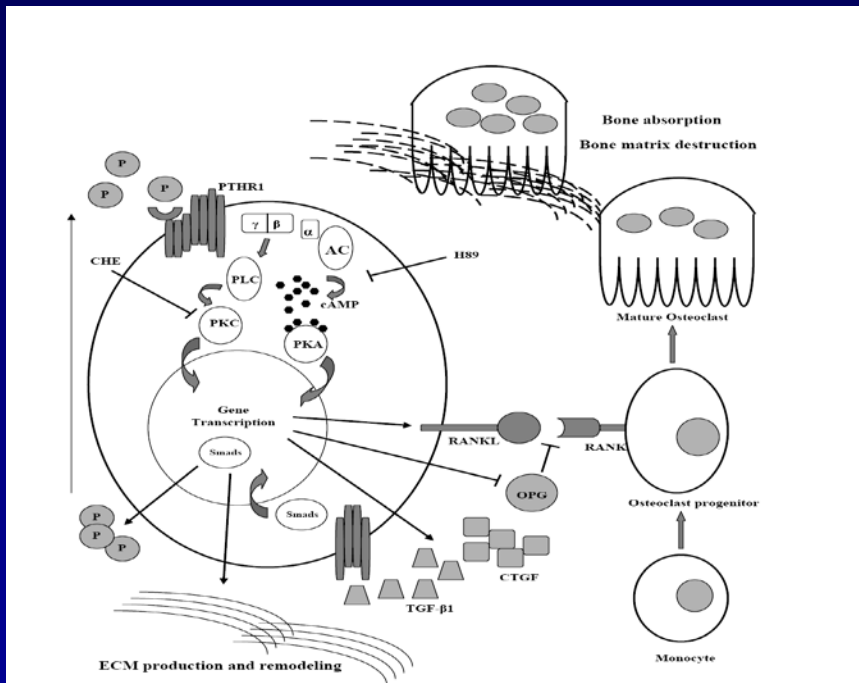
Table 10.4: Cancer of the Bone and Joint: Number and Dist 8-, & 10-Year Relative Survival Rates (%) by Histology, Ages

Histology	Cases	Percent	Median Survival (Months)
Total	2,273	100.0	>120
Ewing Sarcoma	187	8.2	59.0
Osteosarcoma	625	27.5	84.5
Chondrosarcoma	944	41.5	>120
Other Histologies	517	22.7	106.6

Bone Tumors in individuals over 20 has a 4X higher incidence overall as compared to less than 20

Another Opportunity for Biology to Define the next Phase 3 Clinical Trial Would be through Utilization of Agents which Disrupt Tumor – Environment Interactions

Schema for osteosarcoma-osteoclast interactions



Yang R, et al, Int J Cancer 121: 943, 2007

Zoledronic Acid (mg/m²/dose [max])

Level 1	1.2 mg/m ² /dose [2 mg]
Level 2	2.3 mg/m ² /dose [4 mg]
Level 3	3.5 mg/m ² /dose [6 mg]
Level 4	4.6 mg/m ² /dose [8 mg]

R A N D O M I Z E	Cycle	1		2		3		4		5		6								
	Week	1	2	3	4	5	6	7*	8	9	10	11	12	13*	14	15	16	17	18	
IE+D:	I	E	D	D	D	I	E	D	I	E	D	I	E	D	I	E	D	I	E	D
OR																				
IE:	I	E			I	E			I	E			I	E			I	E		

I=Ifosfamide 2.8 gm/m²/d x 5 days
 E=Etoposide 100 mg/m²/day x 5 days
 D=Denosumab 120 mg or 3 mg/kg SQ
 S=Surgery

* Cycles after surgery begin after adequate post-surgical healing has occurred.
 ▼ Tumor assessment

Conclusions

- Osteosarcoma is characterized by genetic complexity and lacks a precursor lesion to help understand the steps in its development.
- Past studies have failed to identify reproducible validated prognostic factors. Cooperative efforts focusing on comprehensive assessments of pathways rather than single gene studies may be necessary to identify clinically relevant prognostic factors.
- Preclinical testing is being used as a method to screen for therapeutic leads in osteosarcoma.
- Future phase 3 studies may randomize all patients to a novel therapy independent of histologic response.
- Considerable excitement exists for testing IGF-1R antibodies in sarcomas after several hurdles are overcome.

