

Biobanking of Pediatric Tumors: Some lessons I have learned

Michael A. Grotzer

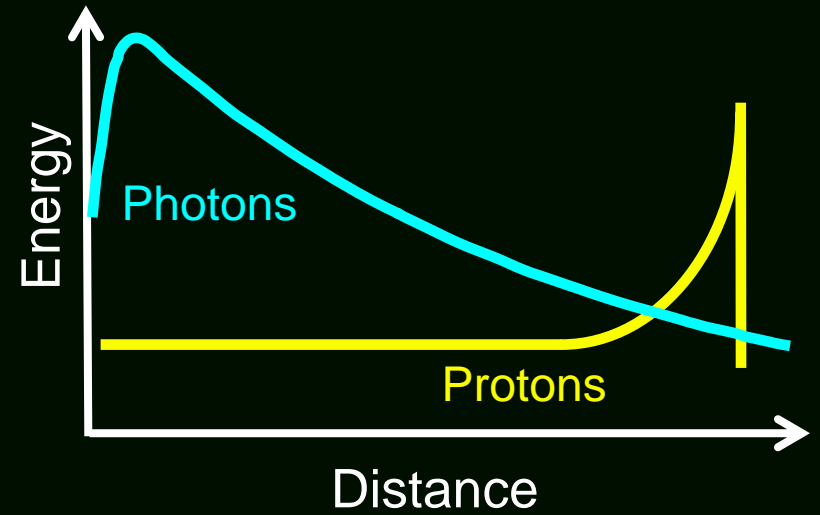
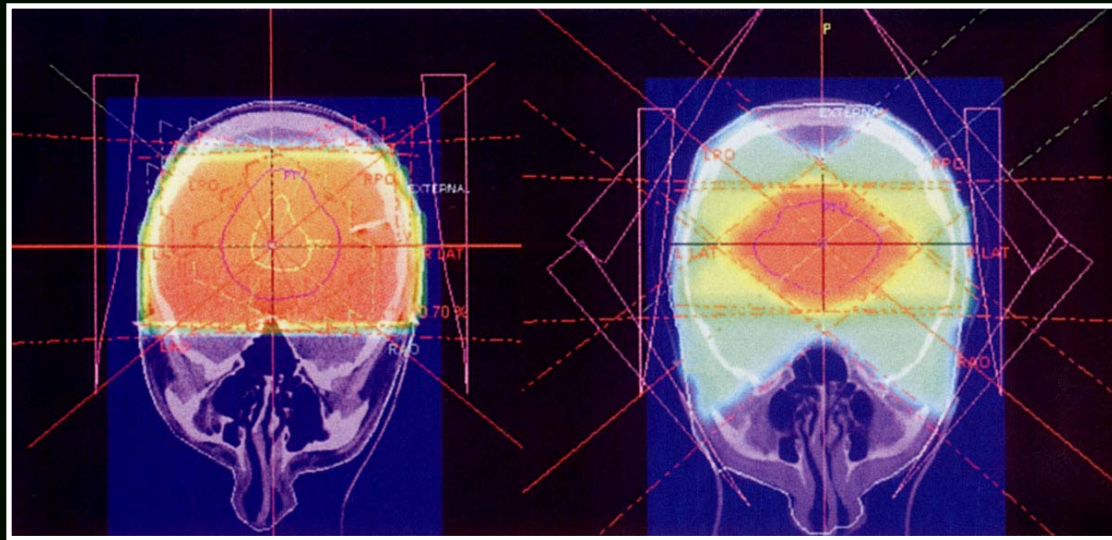
University Children's Hospital of Zurich, Switzerland

Krusenberg Herrgård, Sweden 2008

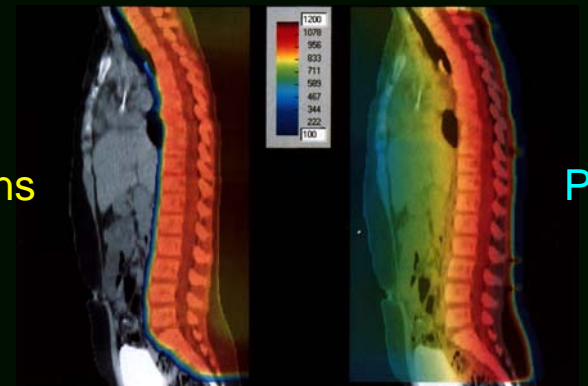
Summery and Conclusions

- Time must be right, motivation must be high
- Think about tumor banking as a research tool
- Solve the legal and ethical issues
 - Owner of the unprocessed tumor sample is the patient
 - Don't try to make money
 - Informed consent, linked anonymization
- Get the local surgeon, pathologist and oncologist on board
- Think about distribution of tumor samples before starting collection
 - Avoid conflicts of interests
- Political problems > logistical problems > technical problems
- Keep it simple

Technical Advances in Radiotherapy

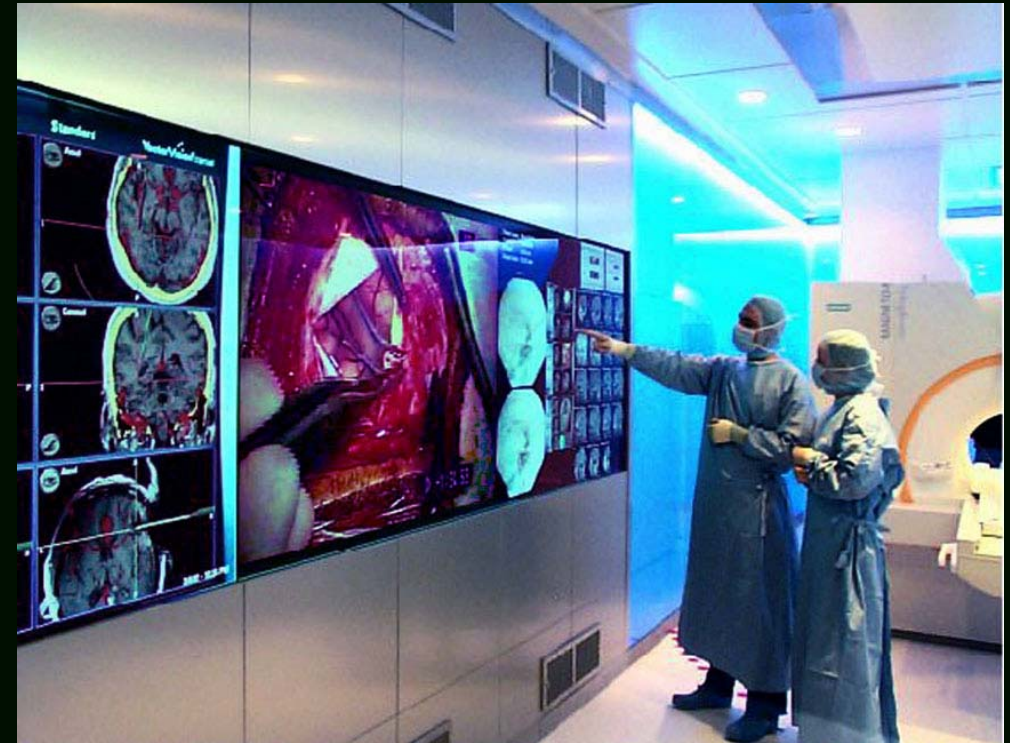
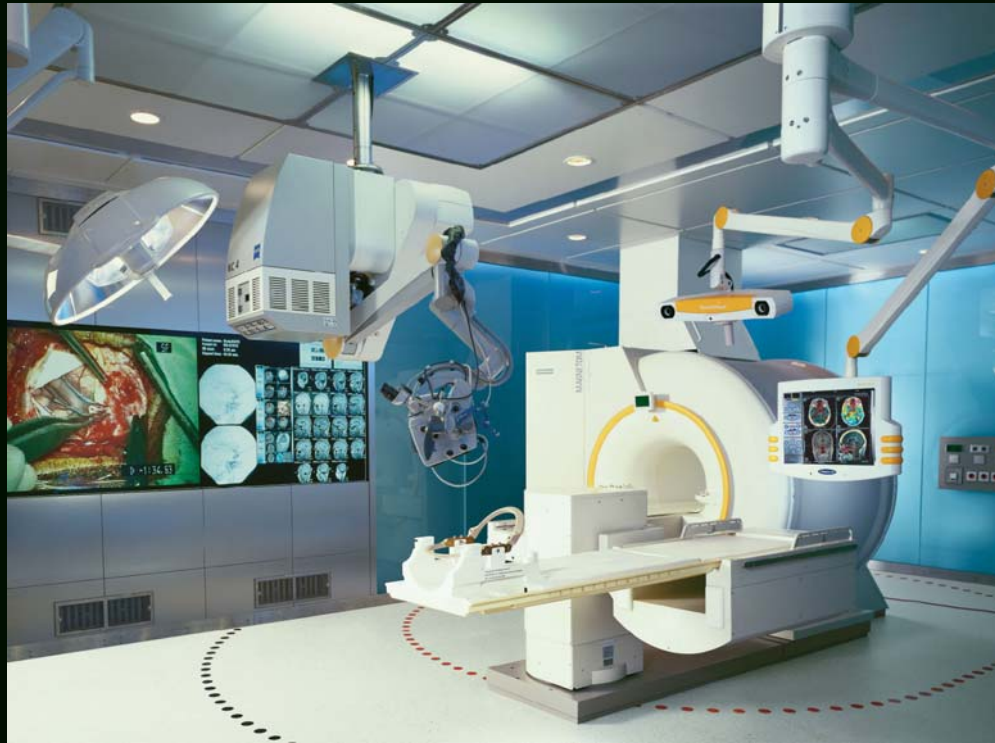


Protons

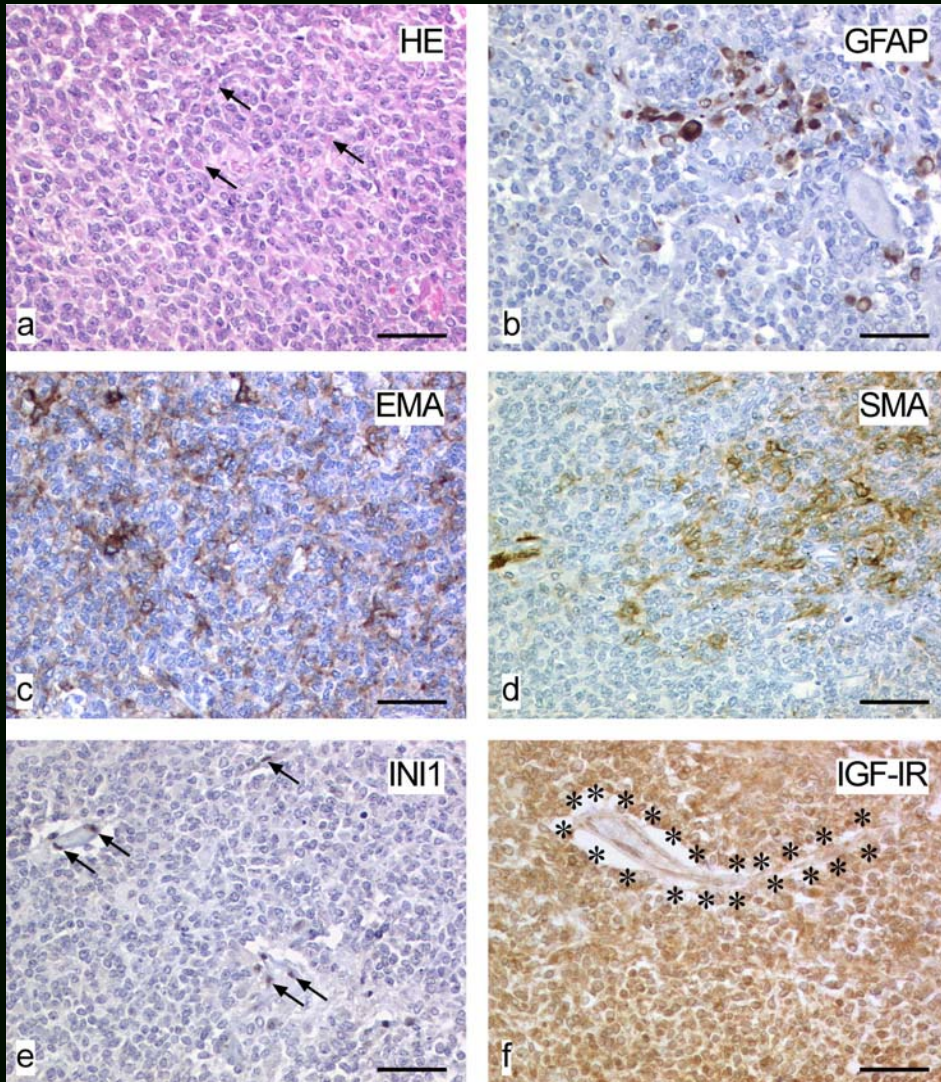


Photons

Technical Advances in Imaging and Neurosurgery



Advances in Neuro-Pathology



Atypical Teratoid/Rhabdoid Tumor
with loss/mutation of INI-1

Longterm Effects of Current Medulloblastoma Therapy



- Growth dysfunction
- Endocrine dysfunction
- Hearing loss
- Alopecia
- Risk for second malignancies
- Social and emotional problems
- Intellectual deficits

Medulloblastoma Risk Stratification

Low Risk



Standard Risk

$\geq 3\text{yr}$
M0
 $< 1.5\text{cm}^2$

10-year survival
50-60%

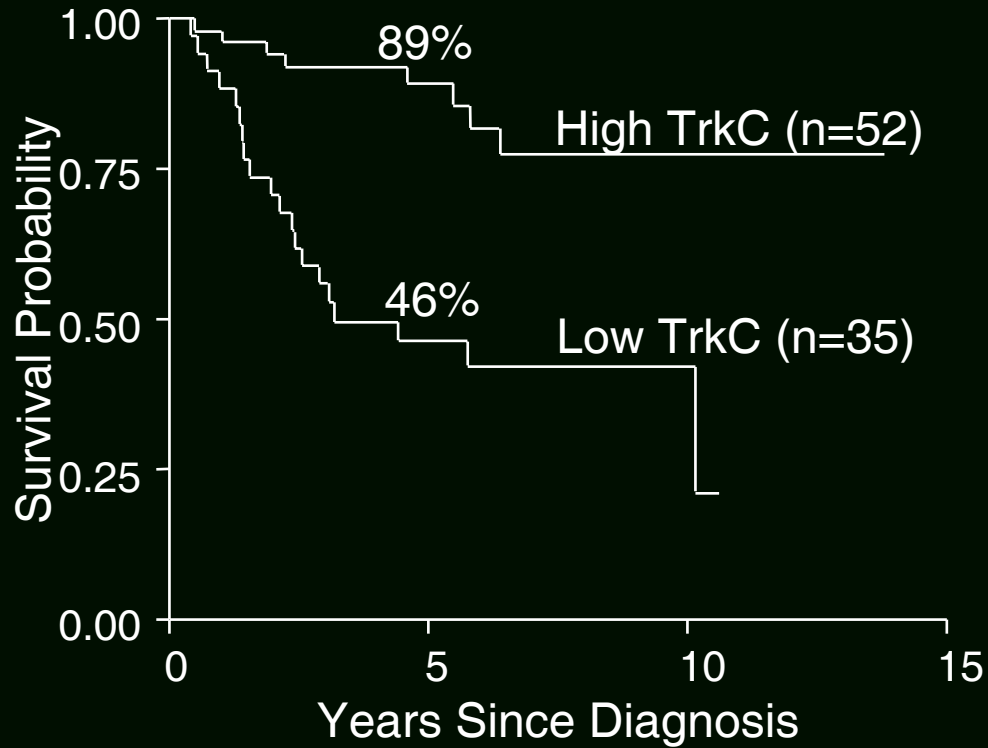
High Risk

$< 3\text{yr}$
M1-4
 $\geq 1.5\text{cm}^2$

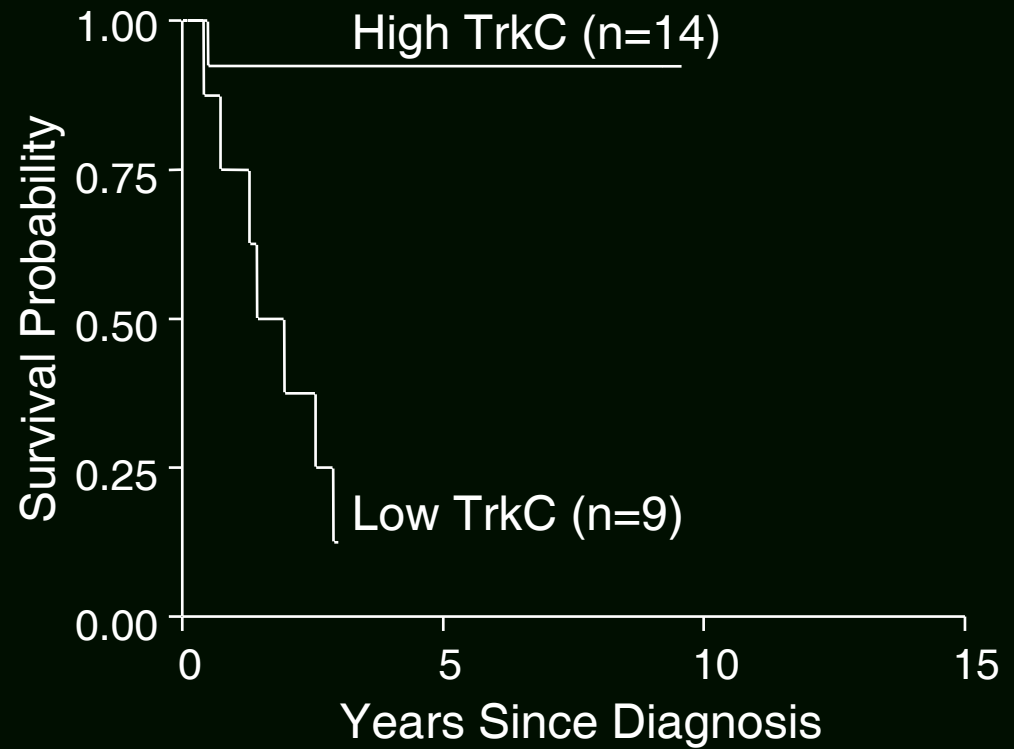
10-year survival
30-40%

TrkC

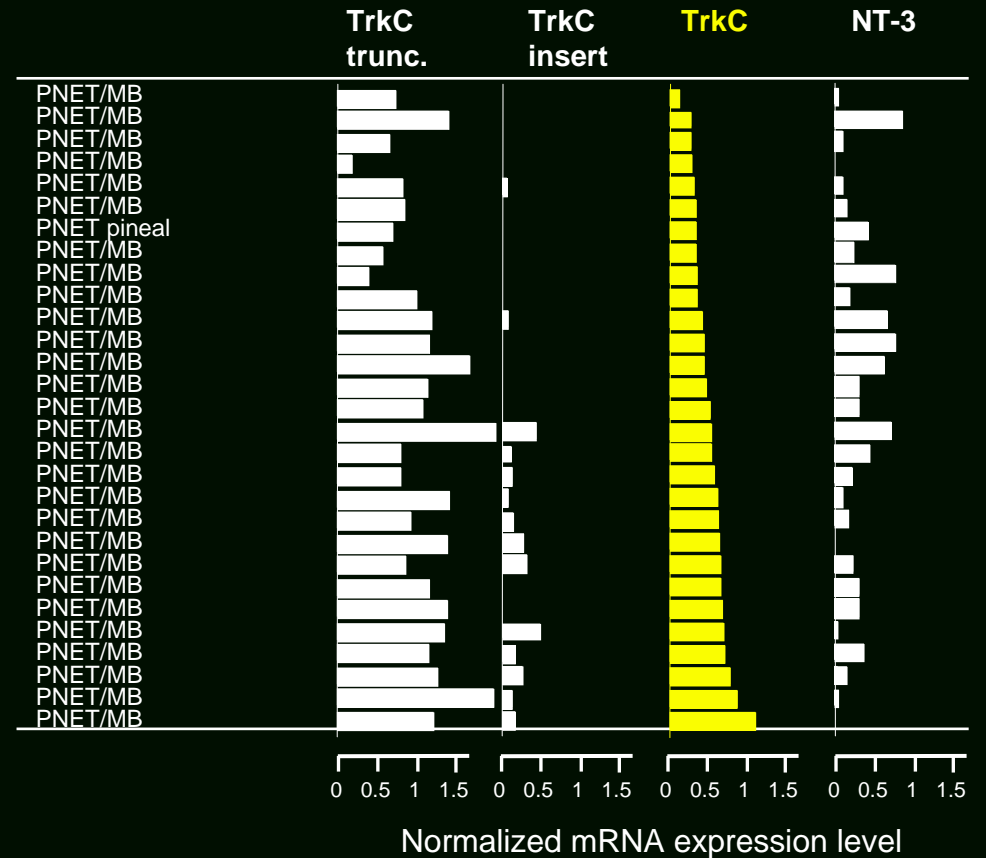
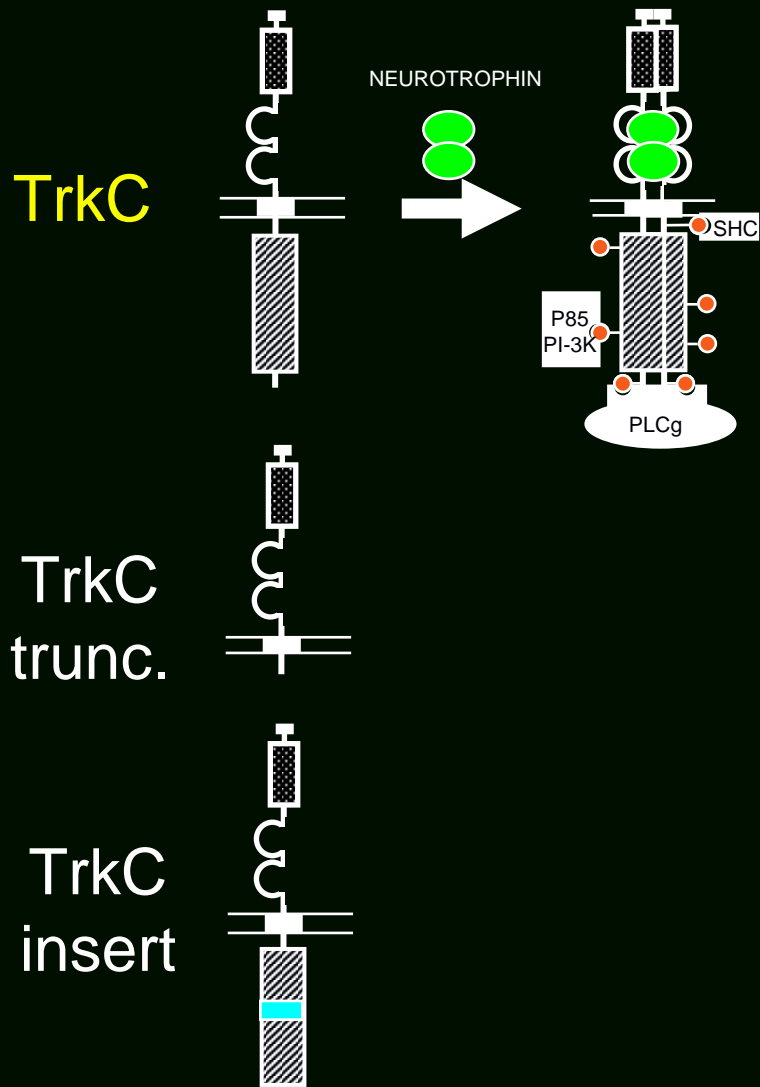
All patients
(n=87)



Patients <3 years at diagnosis
(n=23)



Why Not Doing TrkC Immunohistochemistry?



Difficulties in Getting Fresh-Frozen Tumor Samples From Pediatric Brain Tumor Patients

■ Fresh-frozen

- No large archives available
- Cost intensive
- Complex logistics
- Gold standard for RNA quantitation

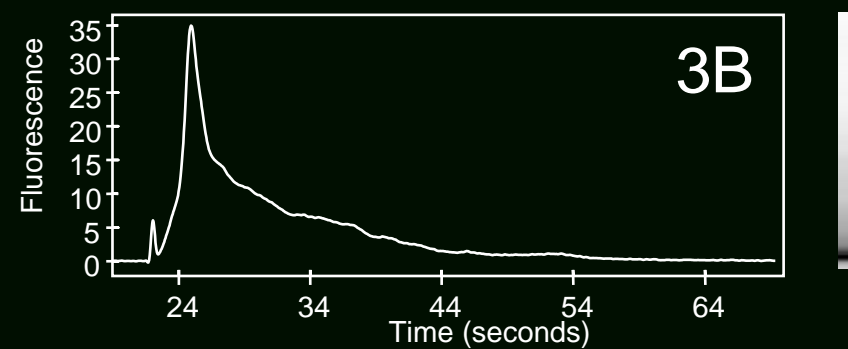
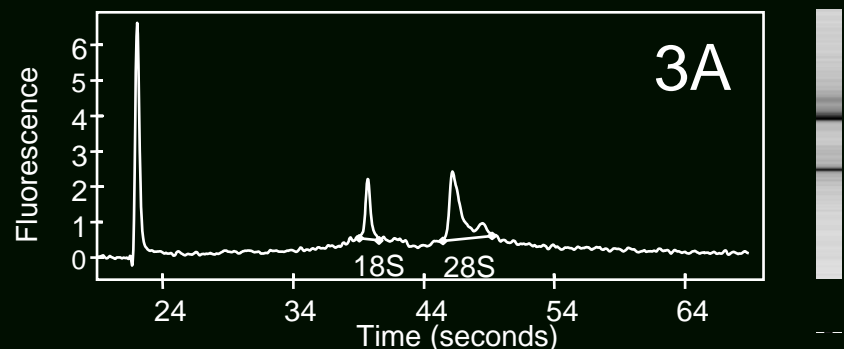
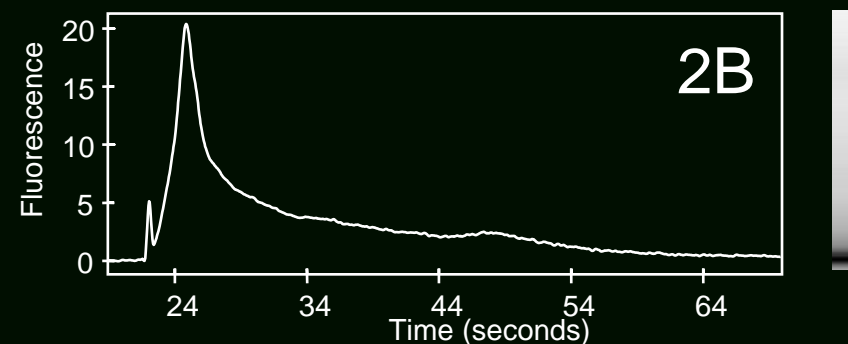
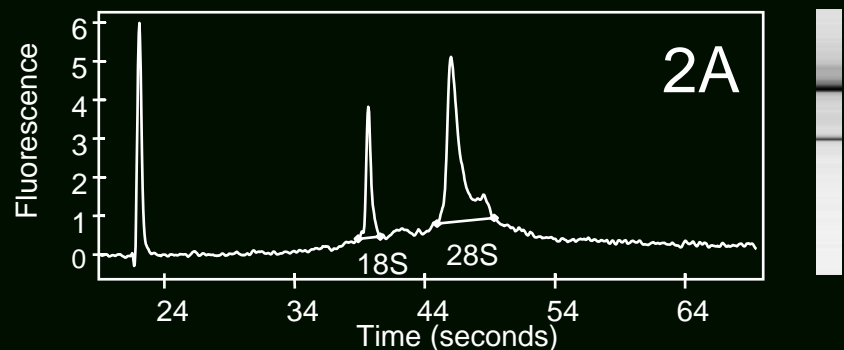
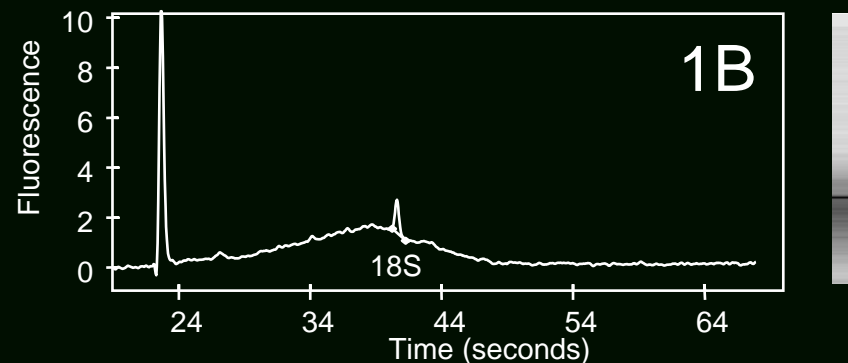
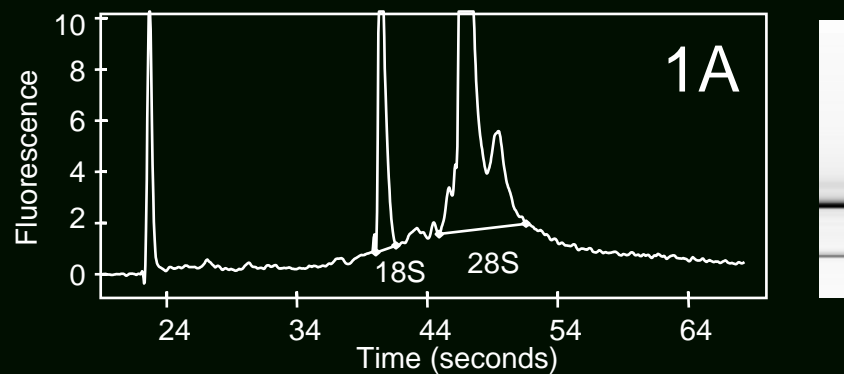
■ Formalin-fixed paraffin-embedded

- Large archives available
- Inexpensive
- No additional logistics needed

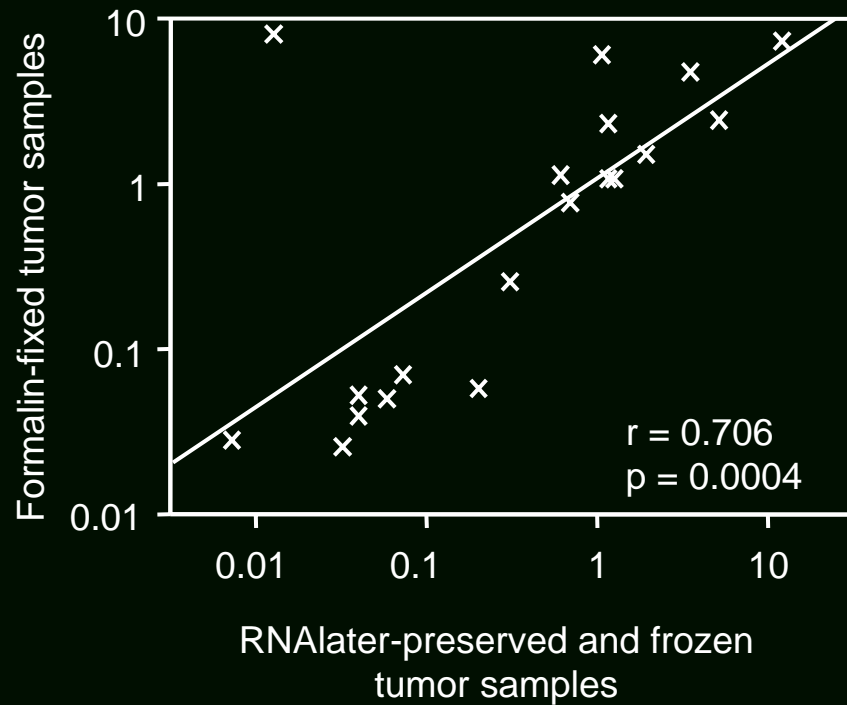
Good enough for RNA quantitation?

Fresh Frozen Tumor Samples

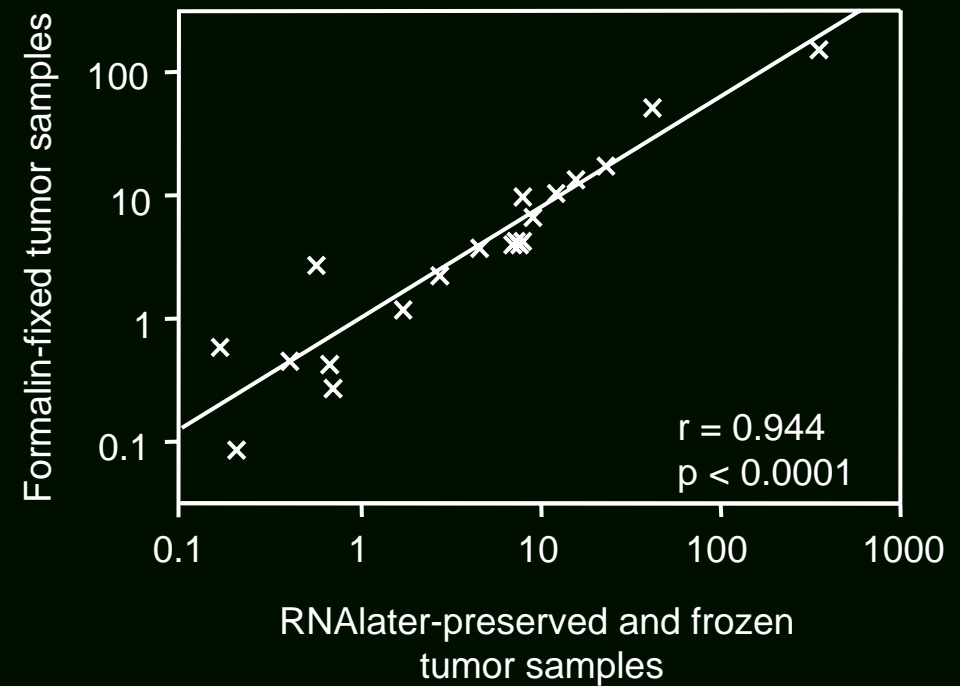
Corresponding Formalin-Fixed Paraffin-Embedded Tumor Samples



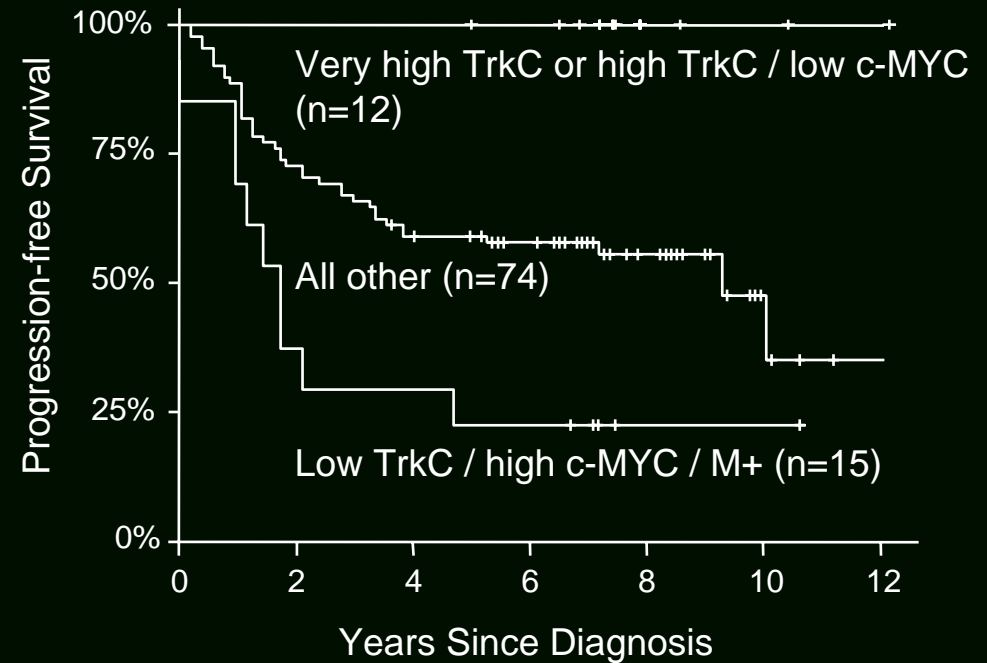
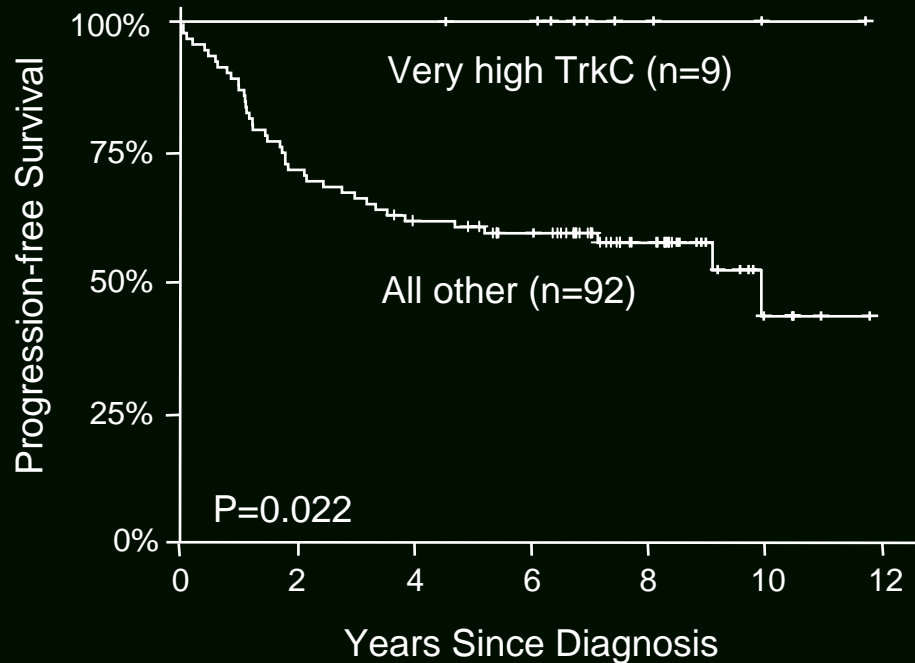
TrkC mRNA expression



c-MYC mRNA expression



Validation Using a Completely Independent Set of 101 Medulloblastoma Tumor Samples



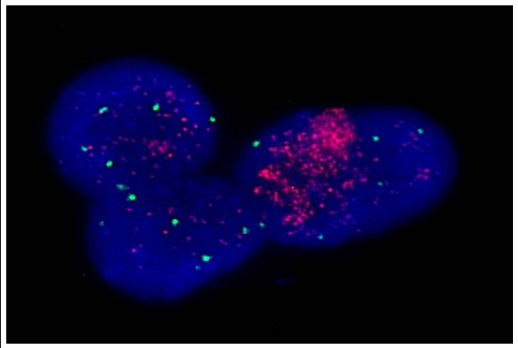
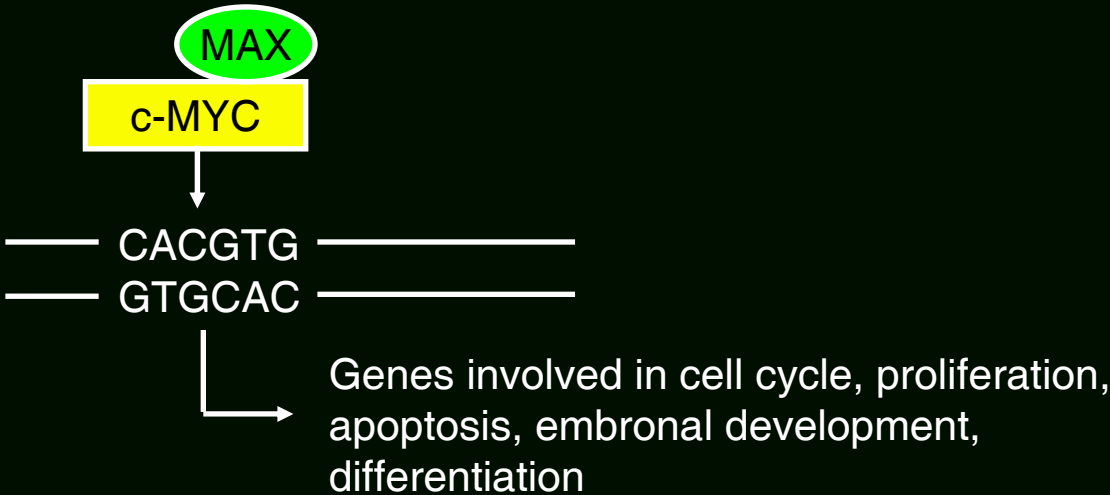
ErbB2

- The unfavorable prognostic significance of the ErbB2 oncogene product (also known as HER2) has been recognized in childhood MB (Gilbertson RJ et al. Br J Cancer 1995, Gajjar A et al. JCO 2004)
- In multivariate analysis, coexpression of HER2 and HER4 demonstrated independent prognostic significance (Gilbertson RJ et al. Cancer Res 1997)

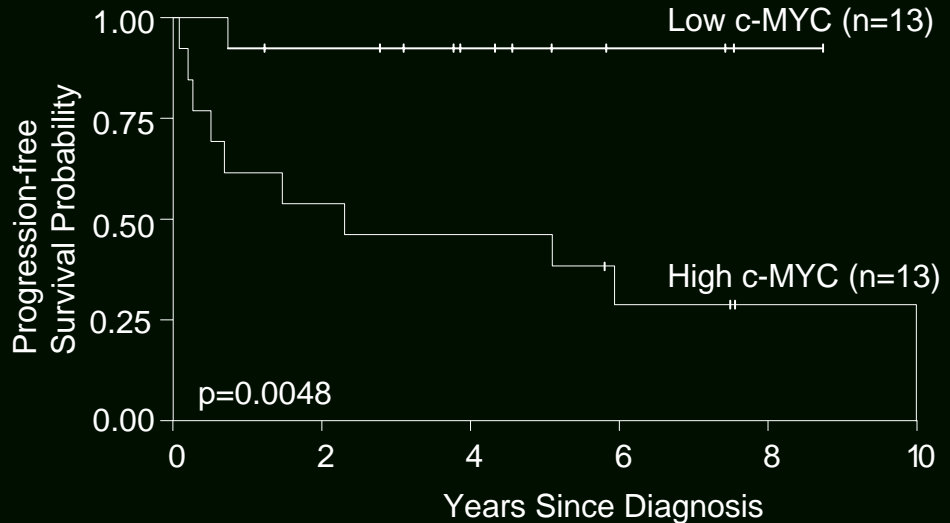
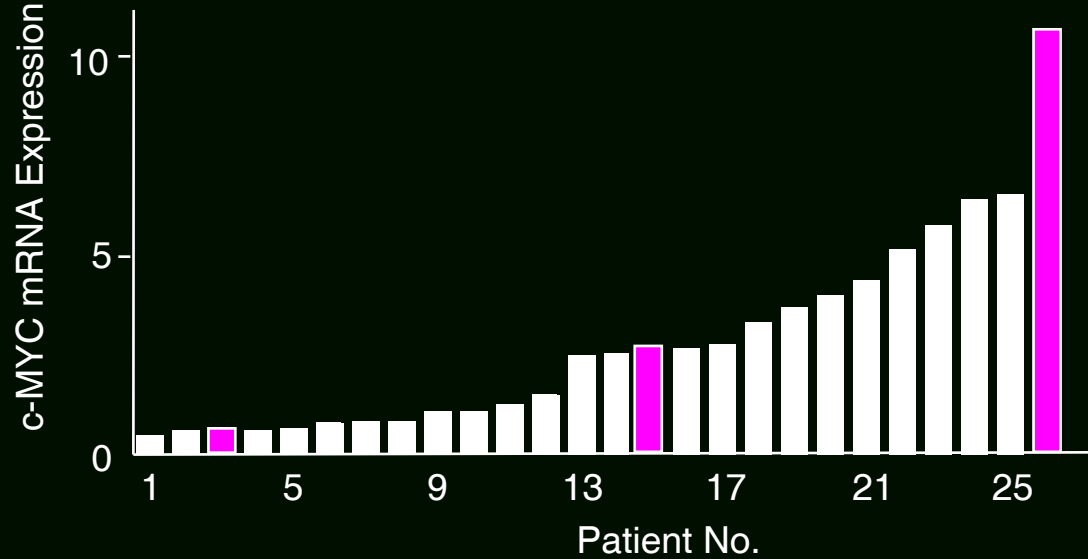
Beta-Catenin

- Children with MB that show a nucleopositive beta-catenin immunophenotype (25%) have favorable survival outcome. Mutation of CTNNB1 were found exclusively among MB that demonstrate nuclear beta-catenin immunoreactivity (Ellison DW et al. J Clin Oncol, 2005)

c-MYC

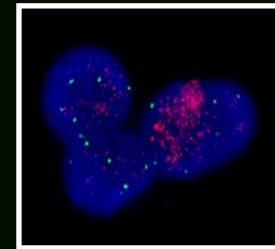
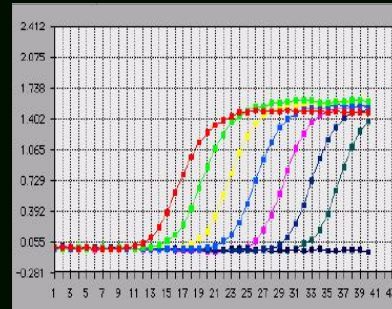


c-MYC amplified

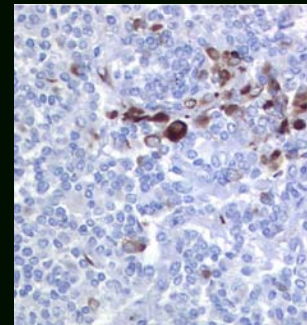
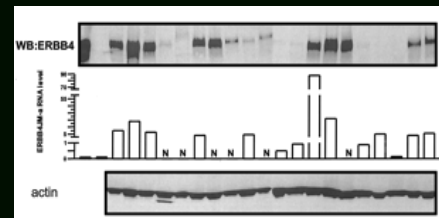


Selecting an Outcome Predictor for Future Clinical Trials

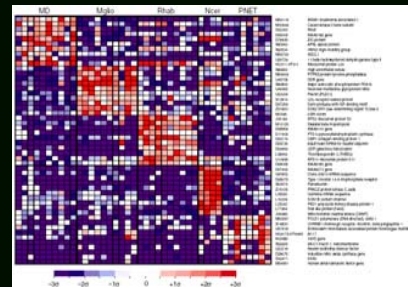
TrkC / c-MYC



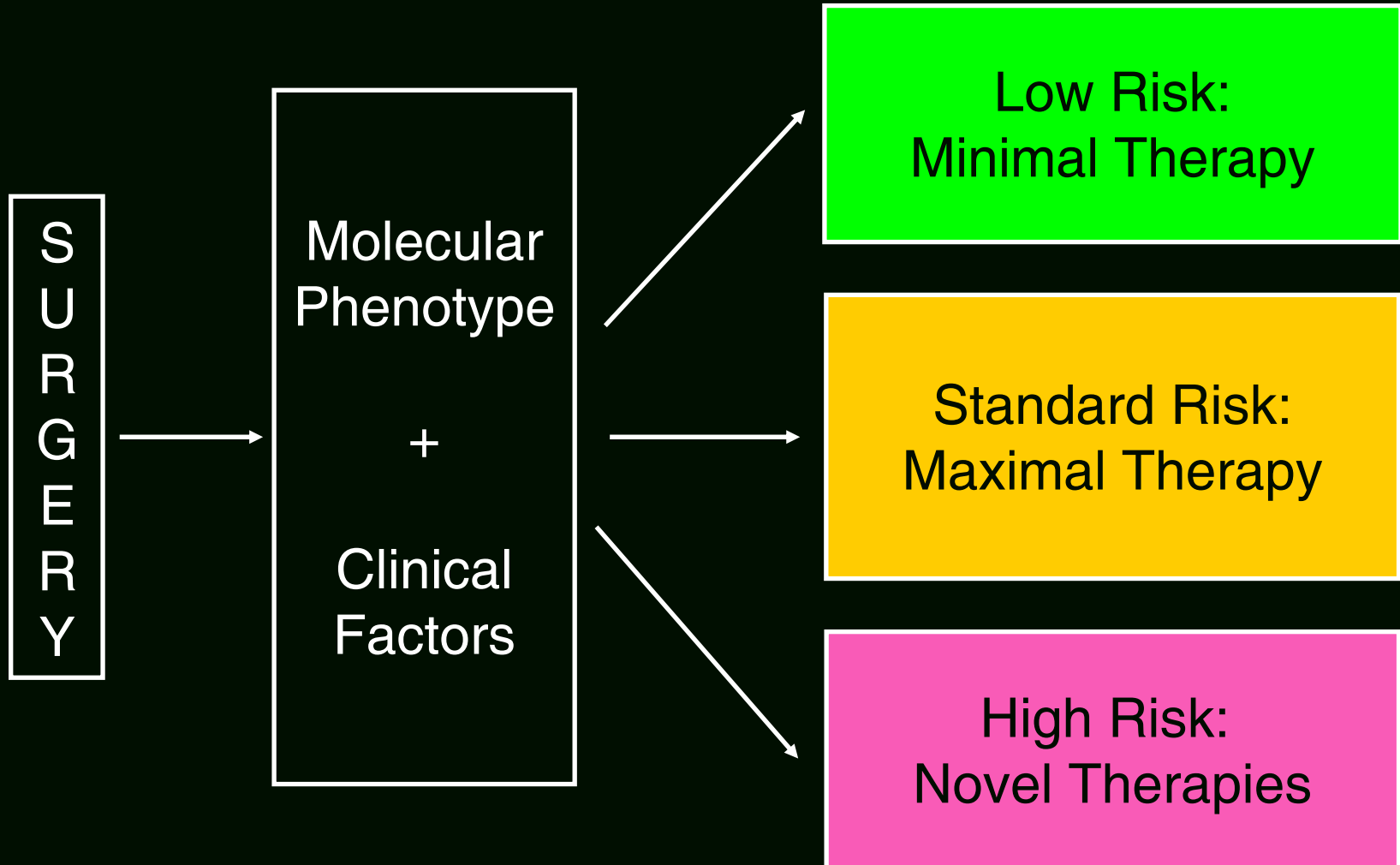
ErbB2 / B4
Beta-catenin



Novel
Markers?



Future Directions



From a better understanding of
childhood tumor biology
more effective
therapeutic strategies
can be developed!

Unlimited Replicative Potential

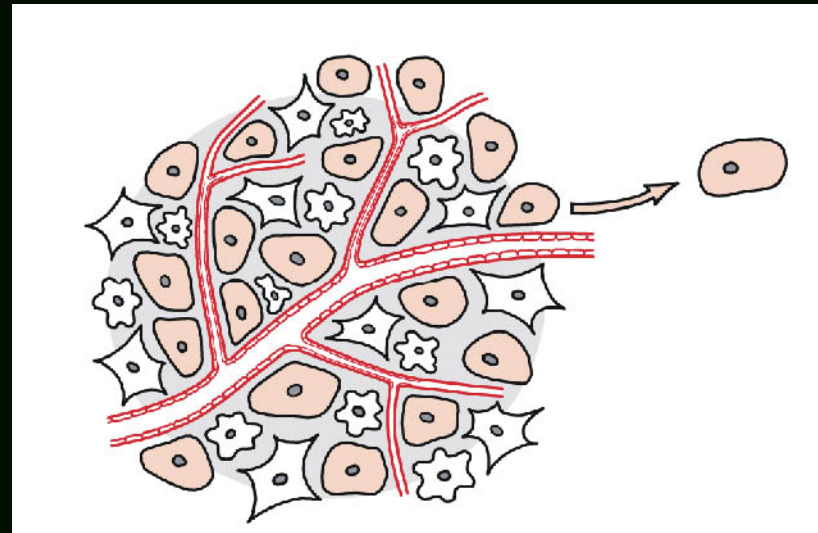
Insensitivity to Antigrowth Signals

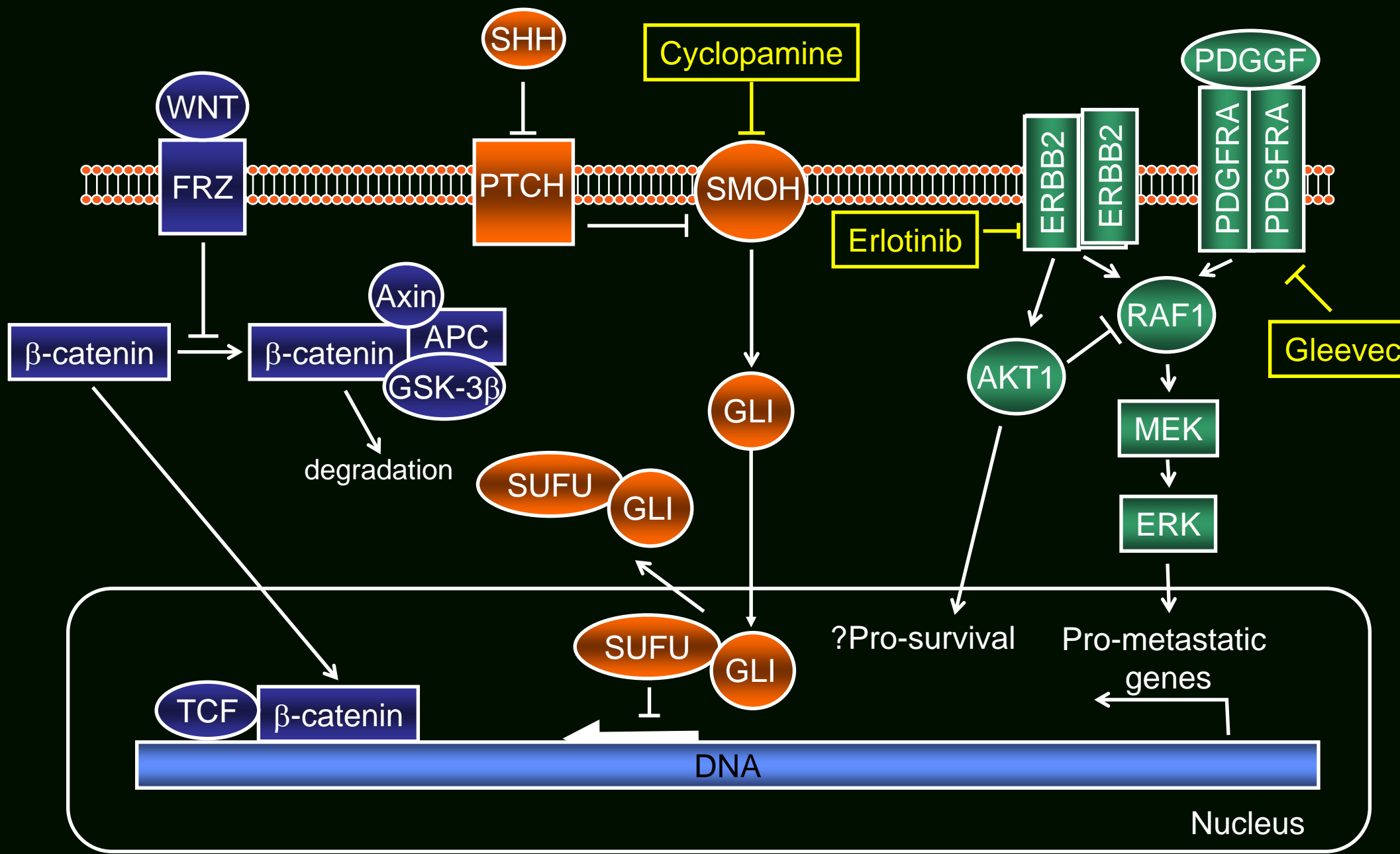
Tissue Invasion & Metastasis

Evasion of Apoptosis

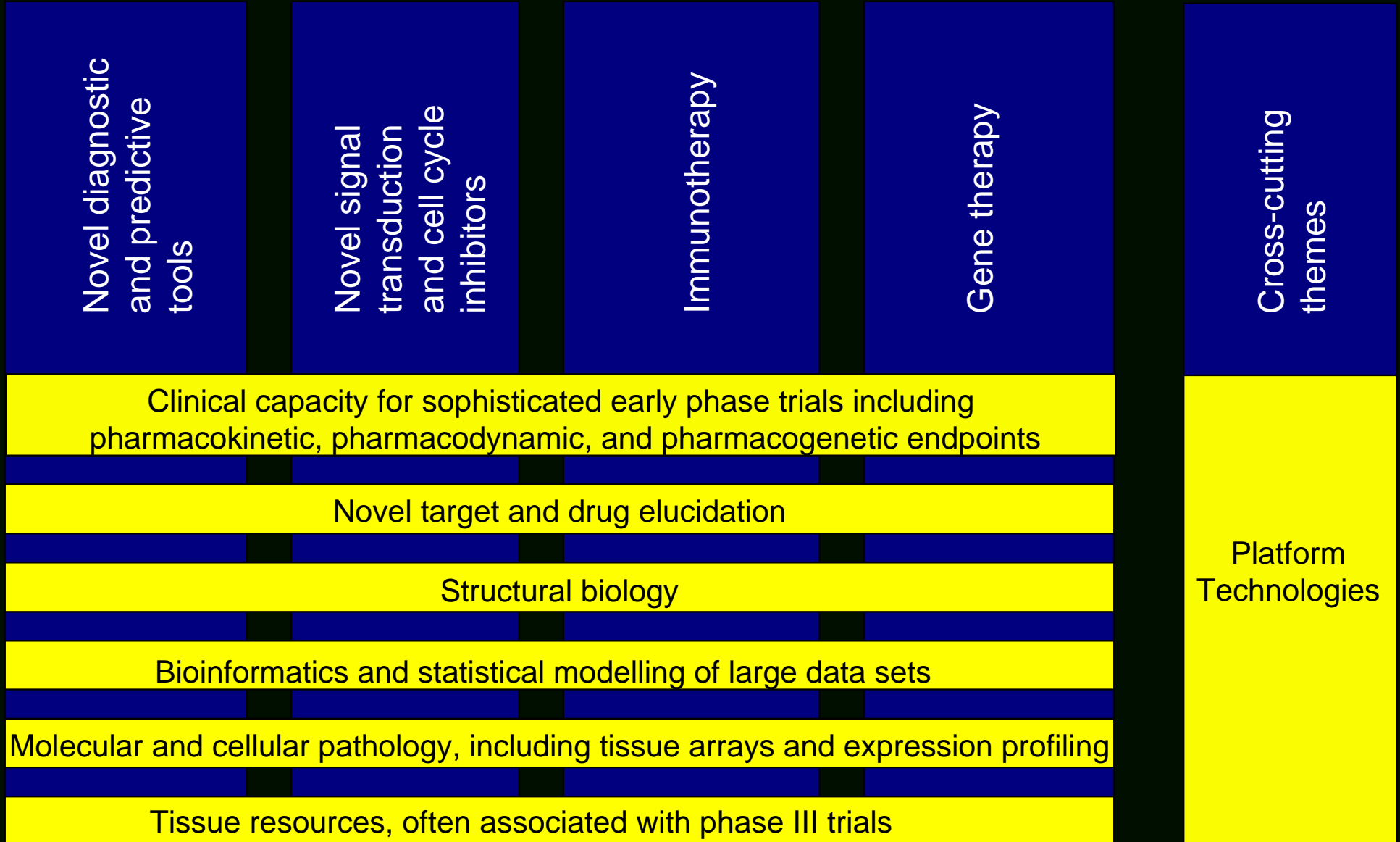
Sustained Angiogenesis

Self-Sufficiency in Growth Signals



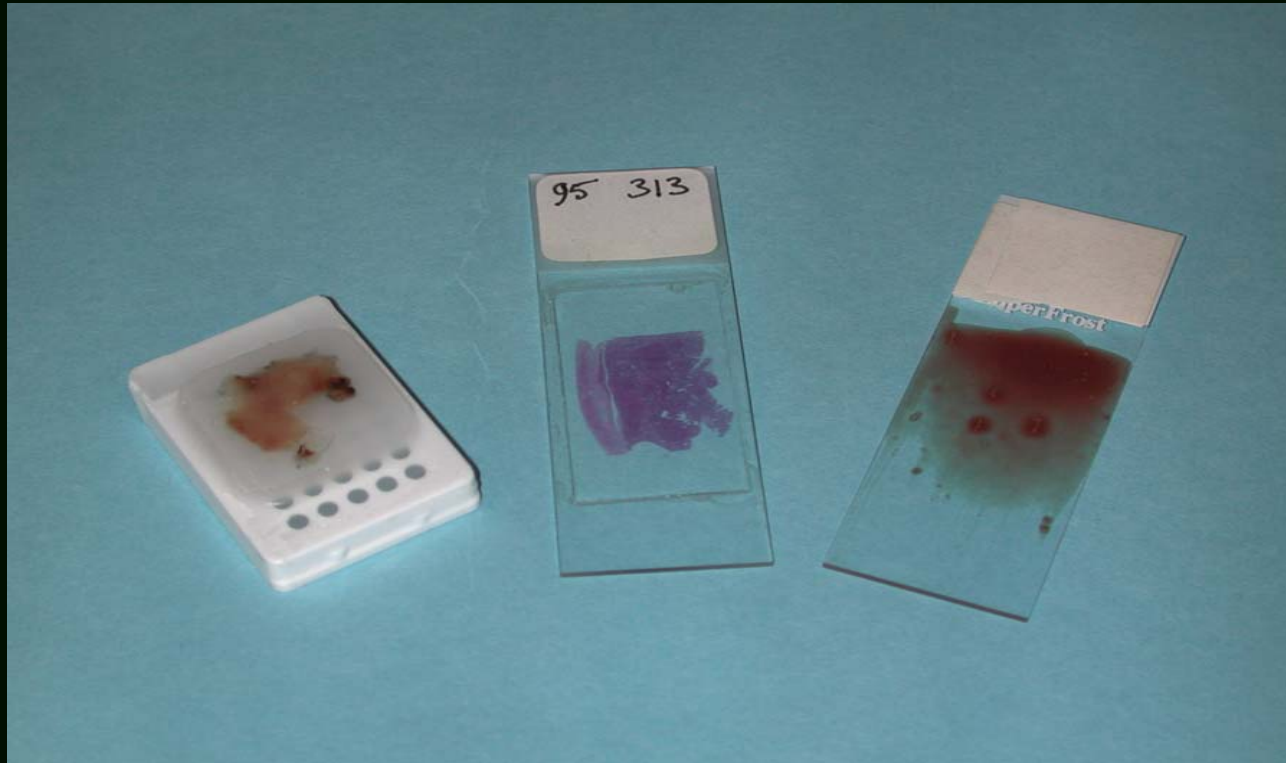


Emerging Translational Research Themes



“Researchers fear that current access to tumor samples is not sufficient to unpick cancer’s secrets” – Nature, 4 April 2002

Rationale to Establish a Tumor Bank of the Swiss Pediatric Oncology Group (SPOG)



Potential Problems of a Tumor Bank

- The veracity of biologic research depends on the quality of the material that is studied
 - Tumor handling and sectioning by the pathologist in the operation theatre
 - Tumor specific guidelines for pathology and biology
- However, maximal standards for handling tumor specimens are not likely to capture the involvement of all centres
- Ethical, legal and management issues

International Society for Biological and Environmental Repositories (www.ISBER.org)

- A Division of the American Society for Investigative Pathology
- ISBER is the leading international forum that addresses the technical, legal, ethical, and managerial issues relevant to repositories of biological and environmental specimens
- Ongoing development of Best Practices guidelines; and provision of centralized information resources for existing repositories
- **ISBER 2008 Annual Meeting: Global Biobanking Collaborations: Challenges and Opportunities. May 18-21, 2008, Bethesda, Maryland, USA**

Kompetenz-Netzwerk GPOH



Tumorbox zur gleichzeitigen Versendung von tiefgefrorenem und nicht gefrorenem Untersuchungsmaterial

GPOH Tumor Bank

- Centralized tissue banks for tumor samples from

>80% of Neuroblastoma patients

<20% of Brain tumor patients

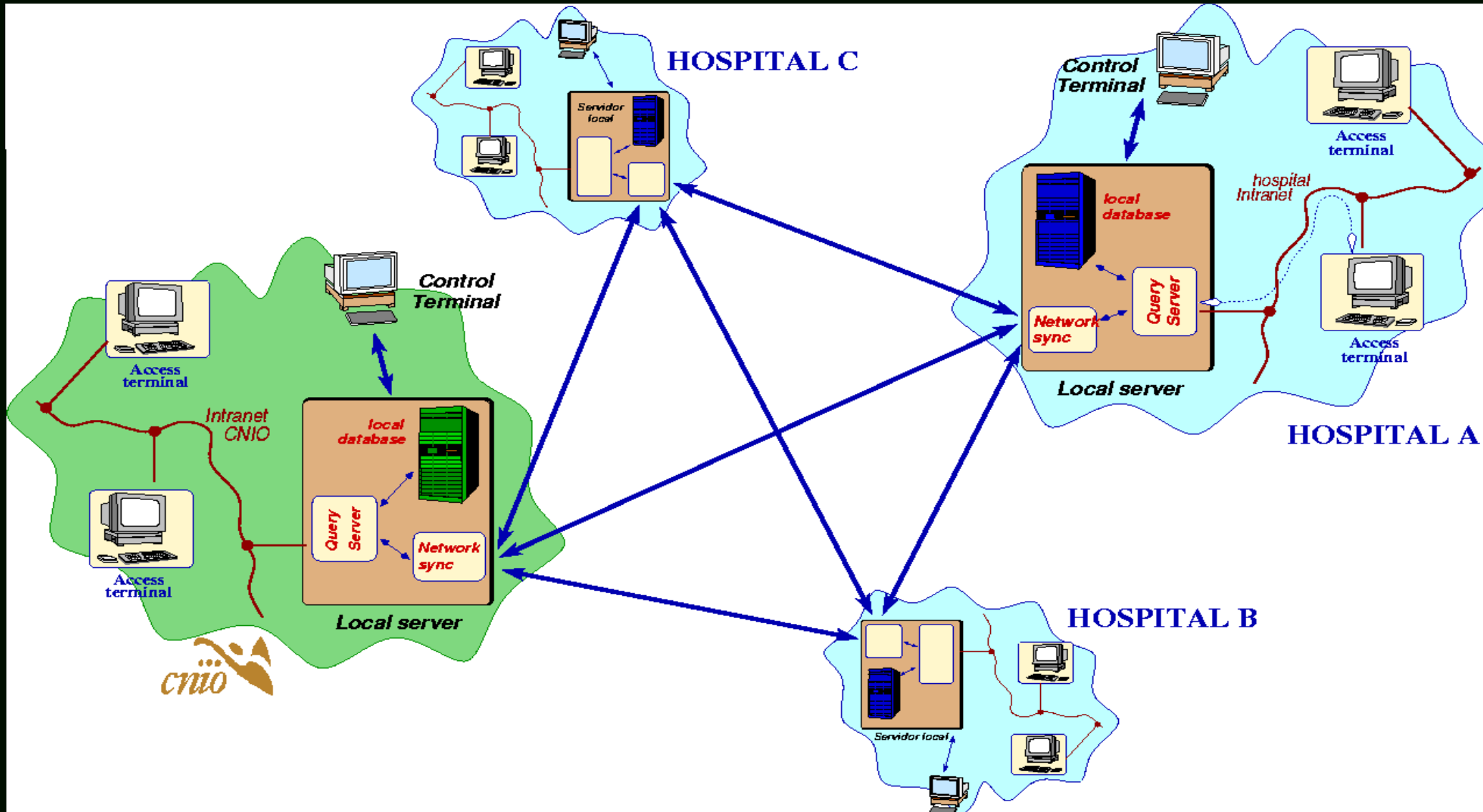
UKCCSG Tumor Bank

Kathryn Pritchard-Jones, December 2002

Tumor type	No. of samples
Neuroblastoma	334
Wilms tumor	278
Rhabdomyosarcoma	106
Lymphomas	183
Germ cell tumors	78
Liver tumors	40
Bone tumors	37
Brain tumors	102

- 21/22 UKCCSG centres have obtained local research ethics committee approval
- 1436 samples registered
- 35 projects approved

Data Management in Tumor Banking



Informed Consent

- ...We are asking your permission to store blood samples and tumor tissue that is not needed for the diagnosis from your child, in a special tumor bank so that they can be made available to scientists carrying out research into the molecular, genetic, immunological and other characteristics of this children's cancerous disease.
- ...The decision to store tumor tissue and blood samples from your child in the Tumor Bank and to make them available for later research investigations is yours. Whatever your decision, it will not affect the care and treatment of your child. Even if you decide to allow tumor tissue and blood samples from your child to be stored and used for research investigations, you can at any time change your mind and inform the doctor who is treating your child accordingly. He or she will then arrange for the stored tumor and blood samples to be destroyed.
- ...Should your daughter/son at this point in time not be legally competent to make this decision, on reaching the age of 16 years they will be contacted by the doctor responsible and asked for their agreement to the continued storage of tumor and blood samples for scientific investigation.

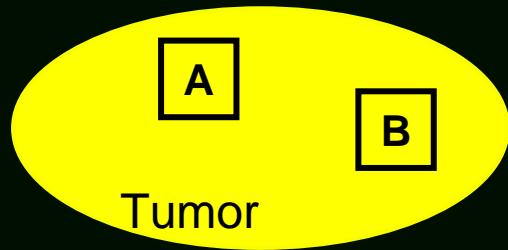
Suspected tumor



Informed consent
by parents

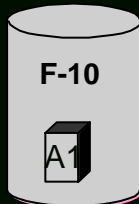


Tumor handling and sectioning in the operation theatre
by trained pathologist / oncologist / surgeon

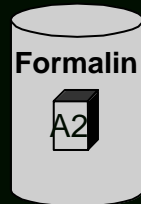


A1 A2 A3

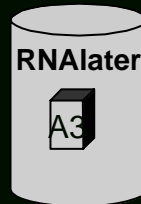
B1 B2 B3



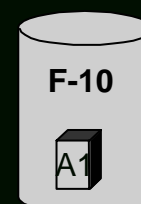
Primary cultures
Permanent cell lines



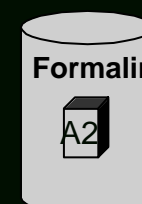
Pathology



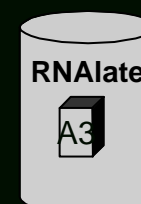
RNA, cDNA
DNA



Primary cultures
Permanent cell lines



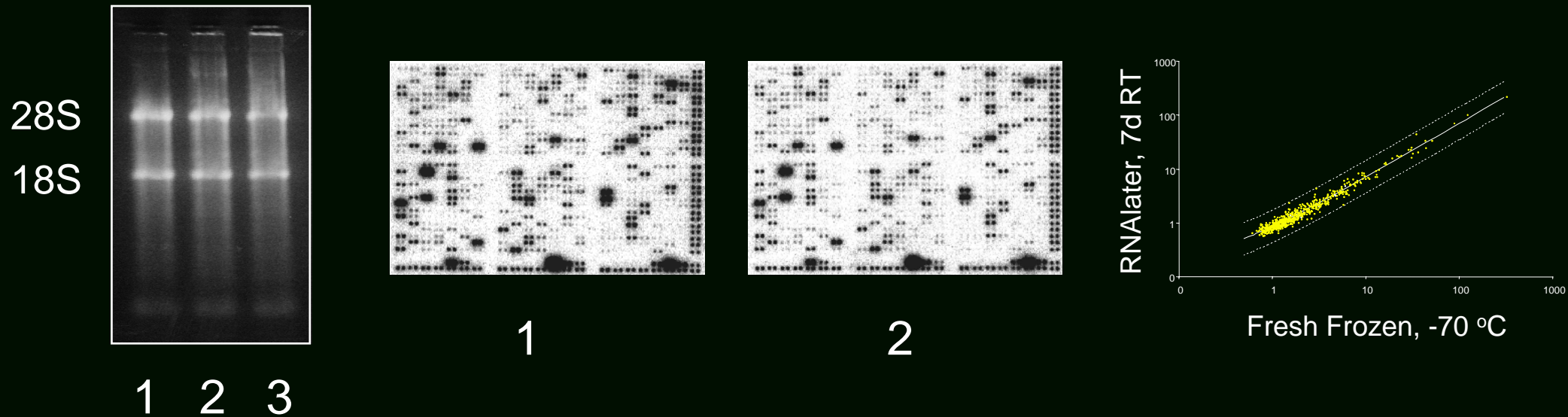
Pathology



RNA, cDNA
DNA

RNA Stability of DAOY Xenograft Tissue

(Grotzer et al. Med Ped Oncol 2000;34:438-42)

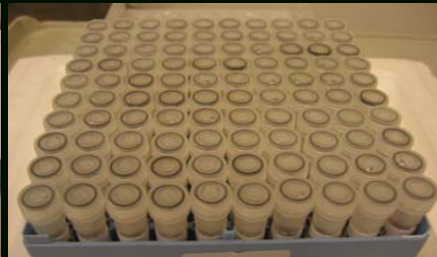
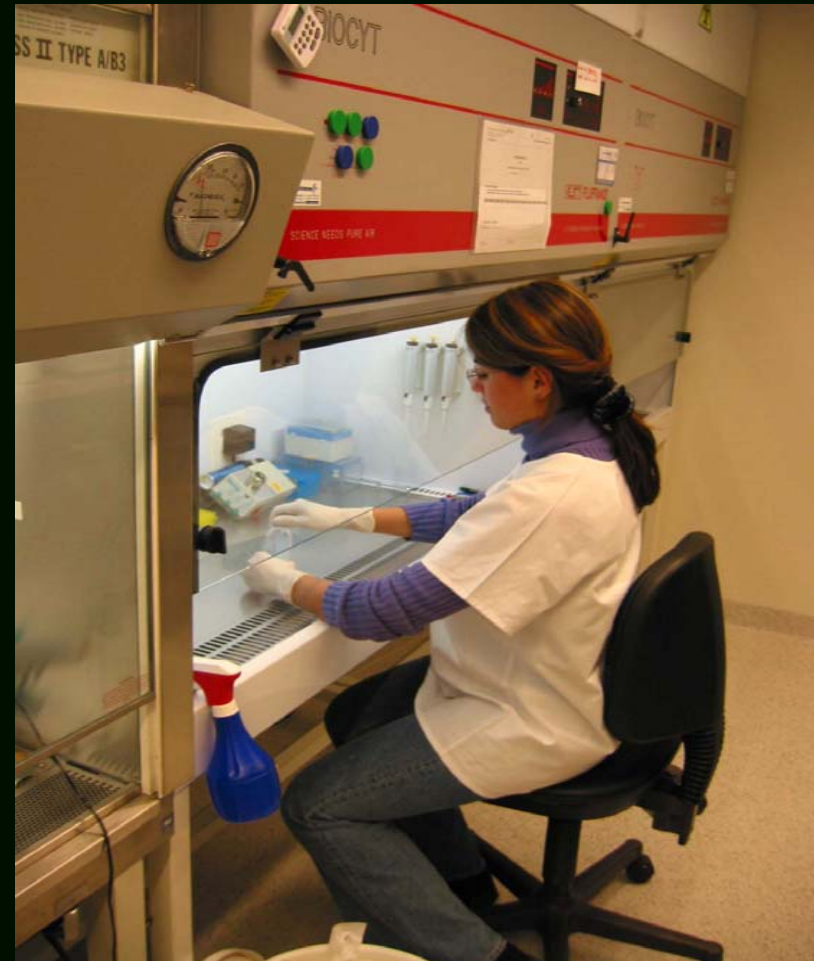


1: snap frozen, -70 °C

2: 7 days at 22 °C (in *RNA later*)

3: 1 day at 37 °C and 6 days at 22 °C (in *RNA later*)

2003-07 Progress Report:
291 samples registered
84 brain tumors
2 projects approved



Linked Anonymization

- The patient's samples will be made anonymous by coding through the treating oncologist before being sent to the tumor bank (Medical Research Council Ethics Services; www.mrc.ac.uk)
- The tumor bank runs a data bank containing a minimal amount of clinical data (age of patient at diagnosis, histology, stage of tumor, therapy given before tumor removal)
- The purpose of this data bank is to help the SPOG research council to decide if a research question can be answered with the material available

- Tumor material will be distributed free of charge for research projects conducted by SPOG-associated scientists.
- Distribution of tumor material will be decided by the research council of the SPOG.
- Proposals should be not longer than 5 pages in length and include:
 - title of project, investigator and affiliation
 - specific aims
 - background and rationale
 - methods and technical feasibility
 - preliminary data
 - statistical considerations
 - funding available to complete the proposed study

Review Criteria of the SPOG Scientific Committee for Access to Specimens

- Will the study move the field forward; is it unique?
- Does the study require the resources of a cooperative group?
- Does the investigator have appropriate expertise/ preliminary data?
- Can the work be done in a timely fashion?
- Will the results of the study have an impact on patient care?
- Does the investigator have funding to conduct the work?

SPOG Tumor Bank

Tumor group	2003	2004	2005	2006	2007	Total
Bone	3	9	3	2	1	18
CNS	16	18	26	13	11	84
Germ cell	-	2	-	1	4	7
Kidney	6	9	6	11	5	37
Liver	-	3	-	-	2	5
Lymphoma	7	6	5	10	9	37
Neuroblastoma	5	9	4	6	6	30
Normal	2	5	2	-	-	9
Rhabdomyosarcoma	2	3	3	2	3	13
Other	10	11	12	11	7	51
Total	51	75	61	56	48	291

2 projects approved

SPOG Tumor Bank

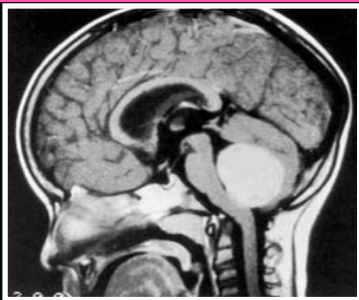
Origin	2003	2004	2005	2006	2007	Total
Aarau	2	-	-	-	-	2
Bern	10	21	10	1	2	49
Basel	-	2	4	1	2	9
Luzern	-	6	2	1	6	15
St. Gallen	-	-	-	11	10	21
Zürich	39	46	45	39	26	195
Total	51	75	61	56	48	291

SIOPEL Liver Tumor Bank

Histology	2004	2005	2006	2007	Total
HCC		6	3	4	13
Hepatoblastoma	2	16	10	6	34
not specified	-	1	2	5	8
Undiff. Sarcoma	-	1	-	-	1
normal liver	-	-	1	-	1
Total	2	24	16	15	57

Number of Liver Tumor Samples Collected

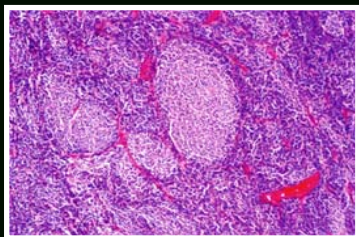
Origin	2004	2005	2006	2007	Total
Australia	-	1	-	-	1
Chile	-		2	3	5
Czech Republic	-	3	2	1	6
England	-	3	-	-	3
France	-	2	2	-	4
Great Britain	-	1	-	-	1
Italy	-	5	2	2	9
Malaysia	-	1	-	-	1
Poland	-	5	4	5	14
Serbia / Montenegro	-	1	-	-	1
Slovakia	-	2	-	-	2
Sweden	-		3	3	6
Switzerland	2	-	-	-	2
The Netherlands	-	-	1	1	2
Total	2	24	16	15	57



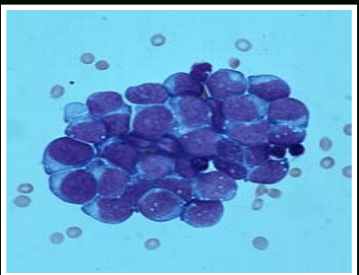
Radiology
Central Review



Analysis of Biological Markers
in Certified Laboratories



Pathology
Central Review

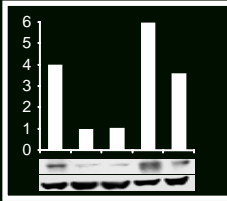
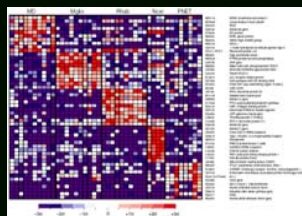


CSF
Central Review

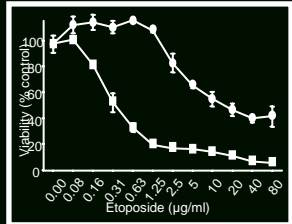
Clinical
Information

Clinical Trial
Center

Local Institution



Clinical
Information



Research Group

Summery and Conclusions

- Time must be right, motivation must be high
- Think about tumor banking as a research tool
- Solve the legal and ethical issues
 - Owner of the unprocessed tumor sample is the patient
 - Don't try to make money
 - Informed consent, linked anonymization
- Get the local surgeon, pathologist and oncologist on board
- Think about distribution of tumor samples before starting collection
 - Avoid conflicts of interests
- Political problems > logistical problems > technical problems
- Keep it simple