

# MICRODISSECTION BASED GENOTYPING

## PLANS FOR PRATT-WHITNEY STUDY



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# MOLECULAR NEUROONCOLOGY

## BASIC PRINCIPLES

- CANCER IS A DISORDER OF GENETIC CONTROLS OF CELL BEHAVIOR
- GENETIC INFORMATION IS ENCODED IN DNA
- CARCINOGENS ACT BY CHANGING DNA
- TUMORS DEVELOP BY ACCUMULATING GENETIC MISTAKES (MUTATIONS)

# HOW GENES CONTROL CELL GROWTH A DELICATE BALANCE

- **ONCOGENES**

  - Signals for cell division

  - Signals for cell movement

  - Signals for formation of blood vessels

- **TUMOR SUPPRESSOR GENES**

  - Stop cell division

  - Death signals

# MALIGNANT BRAIN TUMORS

## MOLECULAR PATHOLOGY

- TYPES OF TUMORS
  - GLIOBLASTOMA
  - MALIGNANT ASTROCYTOMA
  - ANAPLASTIC OLIGODENDROGLIOMA

# GENETIC PROFILING

## DIFFERENT APPROACHES

- MICROARRAY
  - STUDIES VERY LARGE NUMBER OF GENES
  - REQUIRES FRESH TUMOR; SPECIAL HANDLING
  - CENTRAL DATA BANK AT NCI
- MICRODISSECTION
  - USES STANDARD PATHOLOGY SPECIMEN
  - SMALLER NUMBER OF GENES PROFILED
  - LOOKS AT DNA

# GENETIC PROFILING

## DIFFERENT APPROACHES

- PROTEOMICS
  - LOOKS AT PROTEINS
  - LOOKS AT LARGE NUMBER OF PROTEINS

# MICRODISSECTION GENOTYPING

- MICRODISSECTION OF ROUTINE SPECIMENS
  - STERIODACTIC BIOPSIES
  - IDENTIFICATION OF REGIONAL HETEROGENEITY
  - READY MADE TUMOR TISSUE BANK
- PCR AMPLIFICATION
- LOSS OF HETEROZYGOSITY
- SEMIAUTOMATED HIGH THROUGHPUT SYSTEM

# BRAIN TUMOR GENETIC PROFILING

- PATTERNS OF GENETIC CHANGES
  - ONCOGENES TURNED ON
  - TUMOR SUPPRESSOR GENES BROKEN
  - DIFFERENT TUMOR TYPES-DIFFERENT PATTERNS
    - GLIOBLASTOMA
    - MALIGNANT ASTROCYTOMA
    - OLIGODENDROGLIOMA



# MALIGNANT BRAIN TUMORS

## GENES THAT ARE INVOLVED

- GROWTH SIGNALING GENES
  - EGFR
- GROWTH SUPPRESSING GENES
  - PTEN; P53;P21;P13
- DEATH SIGNALING GENES
  - P53

# MICRODISSECTION GENOTYPING UPMCC EXPERIENCE

- PROGNOSTICATION
  - 1p deletion: high treatment response
  - Long term survivors: 1p deleted
  - High and low risk low grades
    - Mutational index: mutated/total informative
      - Fibrillary astrocytomas
      - Pilocytic astrocytomas
      - Pleomorphic xanthoastrocytomas

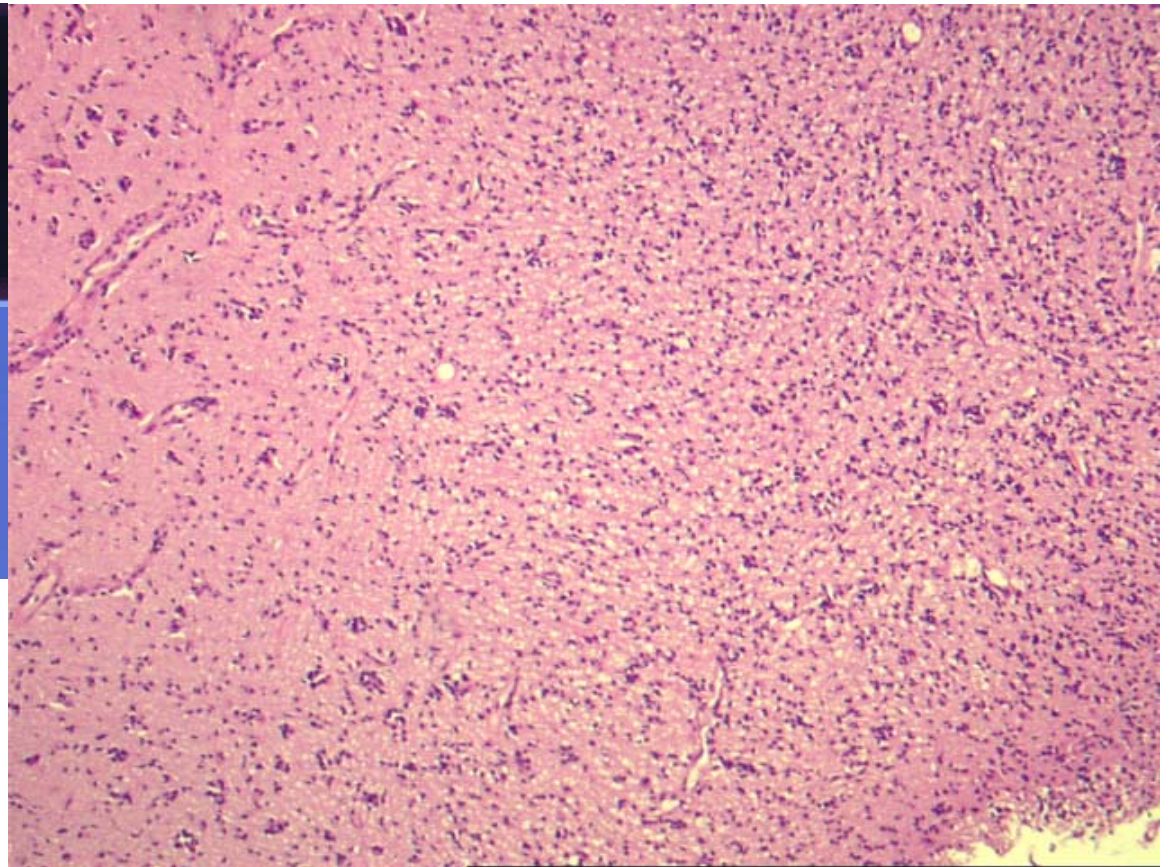
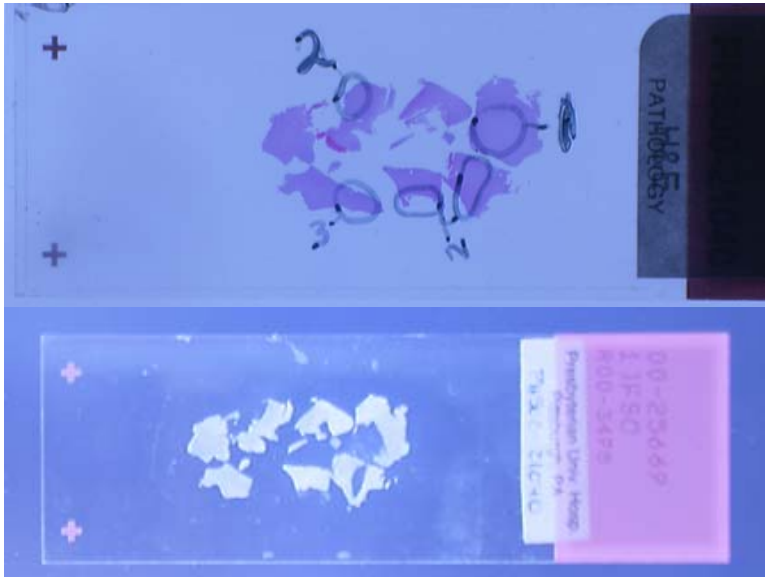
# GLIOBLASTOMA MULTIFORME GENETIC PROFILING

- GENETICS SEPARATE GROUPS
  - CHROMOSOME 1P DELETIONS
  - GROUP 1: NO EGFR MUTATION; MUTATED P53, PTEN LOSS
  - GROUP 2: P53 INTACT; EGFR AMPLIFIED

# MOLECULAR GROUPING OF GBMS

## WHAT WE KNOW

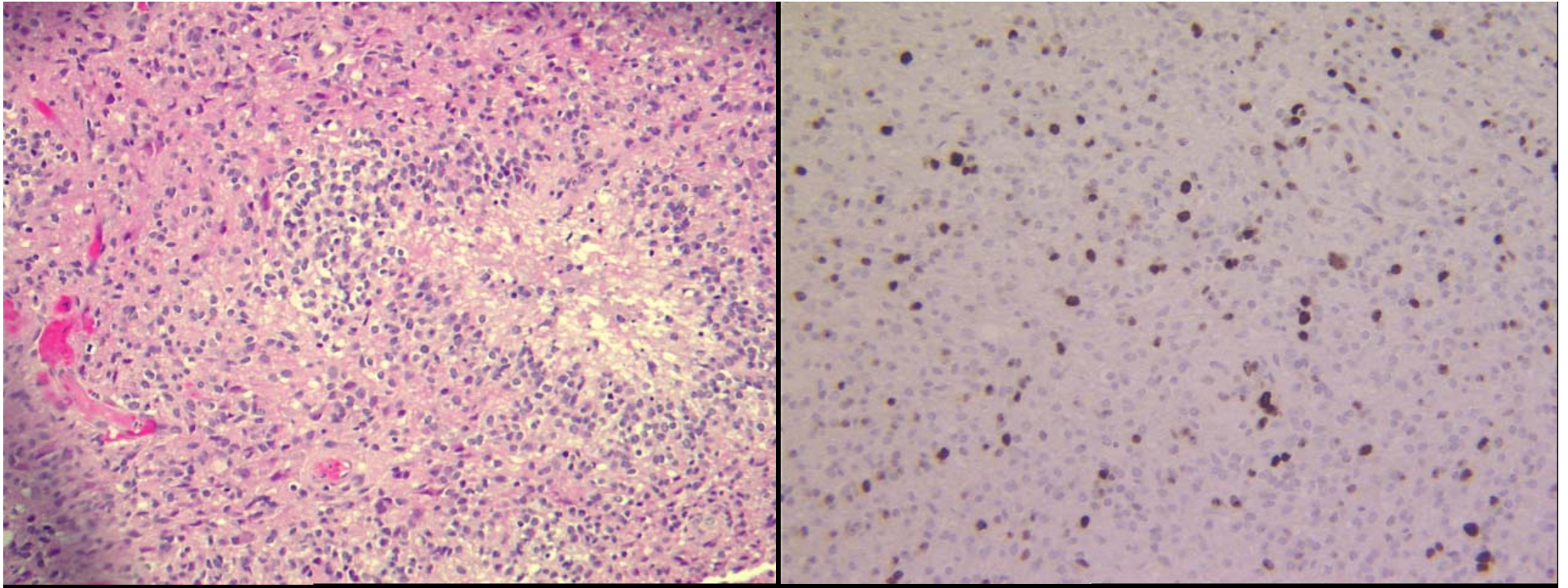
- 1p deletion group much better outcome
- Group 1: younger patients, arise from low grades
- Group 2: older patients, start malignant



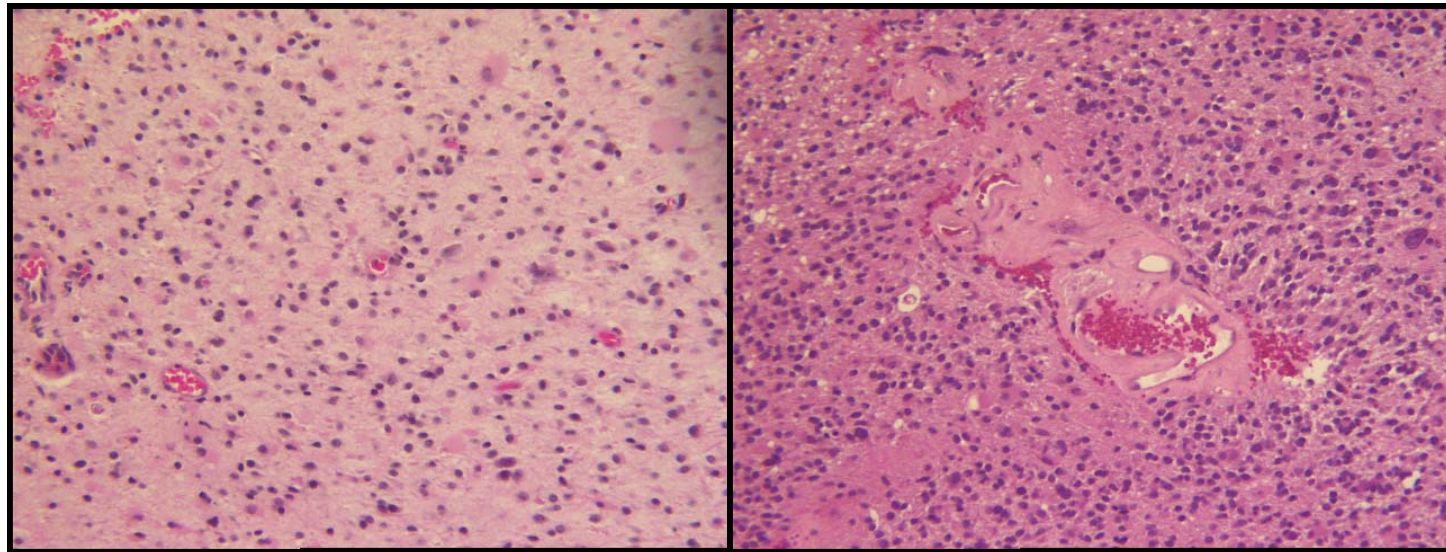
**FRACTIONAL ALLELIC LOSS:**

**# MUTATED MICROSATELLITES DIVIDED BY  
# TOTAL INFORMATIVE MICROSATELLITES**

PHS00-21040	1p D1S 407	1p MYCL	1p D1S 1193	9p21 D9S 254	9p21 D9S 251	10q23 D10S 520	10q23 D10S 1173	17p13 D17S 1163	17p13 TP53	17p13 D17S 974	17p13 D17S 1289	19q D19S 400	19q D19S 559
CORTEX	I	I	I	I	I	I	I	NA	NA	NA	NA	I	I
TUMOR 1	LOH T2	LOH B3	LOH T2	NO LOH	NO LOH	LOH T2	NO LOH	NA	NA	NA	NA	LOH B3	LOH T3
TUMOR 2	LOH T2	LOH B3	LOH T2	NO LOH	NO LOH	NO LOH	NO LOH	NA	NA	NA	NA	LOH B3	LOH T3
TUMOR 3	LOH T2	LOH B3	LOH T2	NO LOH	NO LOH	NO LOH	NO LOH	NA	NA	NA	NA	LOH B3	LOH T3



PHS00-25390	1p D1S 407	1p MYCL	1p D1S 1193	9p21 D9S 254	9p21 D9S 251	10q23 D10S 520	10q23 D10S 1173	17p13 D17S 1163	17p13 TP53	17p13 D17S 974	17p13 D17S 1289	19q D19S 400	19q D19S 559
CORTEX 40% TUMOR	NI	I LOH B2	I LOH B2	NI	I	NI	I LOH B2	I	I	NI	I	NI	I
TUMOR 1	NI	LOH B3	LOH B3	NI	NO LOH	NI	LOH B3	NO LOH	NO LOH	NI	NO LOH	NI	NO LOH
TUMOR 2	NI	LOH B3	LOH B3	NI	NO LOH	NI	LOH B3	NO LOH	NO LOH	NI	NO LOH	NI	NO LOH
TUMOR 3	NI	LOH B3	LOH B3	NI	NO LOH	NI	LOH B3	NO LOH	NO LOH	NI	NO LOH	NI	NO LOH



S96-25088 & S97-724 & PHS00-25415	1p D1S 407	1p MYCL	1p D1S 1193	9p21 D9S 254	9p21 D9S 251	10q23 D10S 520	10q23 D10S 1173	17p13 D17S 1163	17p13 TP53	17p13 D17S 974	17p13 D17S 1289	19q D19S 400	19q D19S 559
96-25088 TUMOR	LOH B3	NO LOH	LOH B2	NI	LOH T3	LOH B3	LOH B2	NA	LOH T3	NI	NI	LOH T3	LOH T3
97-724 TUMOR 1	NO LOH	NO LOH	LOH B2	NI	LOH T3	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3
97-724 TUMOR 2	NO LOH	LOH B2	NO LOH	NI	LOH T3	LOH B2	NO LOH	LOH B2	LOH T2	NI	NI	LOH T3	LOH T3
97-724 TUMOR 3	LOH B2	LOH B2	LOH B2	NI	LOH T3	LOH B3	NO LOH	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3
00-25415 TUMOR 1	NO LOH	NO LOH	NO LOH	NI	LOH T3	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3
00-25415 TUMOR 2	NO LOH	NO LOH	NO LOH	NI	LOH T3	NO LOH	LOH T3	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3
00-25415 TUMOR 3	NO LOH	NO LOH	NO LOH	NI	LOH T3	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3
00-25415 TUMOR 4	NO LOH	NO LOH	NO LOH	NI	LOH T3	NO LOH	NO LOH	NO LOH	NO LOH	NI	NI	LOH T3	LOH T3

# MICRODISSECTION BASED GENOTYPING PRATT-WHITNEY STUDY

- OBTAIN PATHOLOGY SLIDES
- PROFILE TUMOR TYPE
- CARCINOGEN SIGNATURES
  - PATTERNS OF MUTATIONS
    - ANIMAL MODELS
  - MOLECULAR TYPE OF MUTATION
    - CELL CULTURE
    - ANIMAL MODELS



# GLIOMA GENETIC PROFILING TISSUE PROCUREMENT

- BIOPSY OR SURGICAL MATERIAL
- 5 PARAFFIN UNSTAINED SLIDES
- TISSUE BLOCK IF AVAILABLE
- DESIGNED TO PROTECT SPECIMENS
  - ONLY USED IF EXCESS TISSUE
  - IF NO BLOCKS
    - WILL NOT USE SLIDES IF 5 ARE ALL LEFT
    - MAY BE AN ISSUE WITH STEREOTACTIC BIOPSY

# GENETIC CHARACTERIZATION

## WHAT COULD BE DONE WITH CURRENT CASES

- TUMOR SPECIMENS
  - MICROARRAY STUDIES
- BLOOD SAMPLES
  - PHARMACOGENOMICS
  - SERUM MARKERS
  - CIRCULATING TUMOR CELLS