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

MICRODISSECTION GENOTYPING

- MICRODISSECTION OF ROUTINE SPECIMENS
  - STERIOTACTIC 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

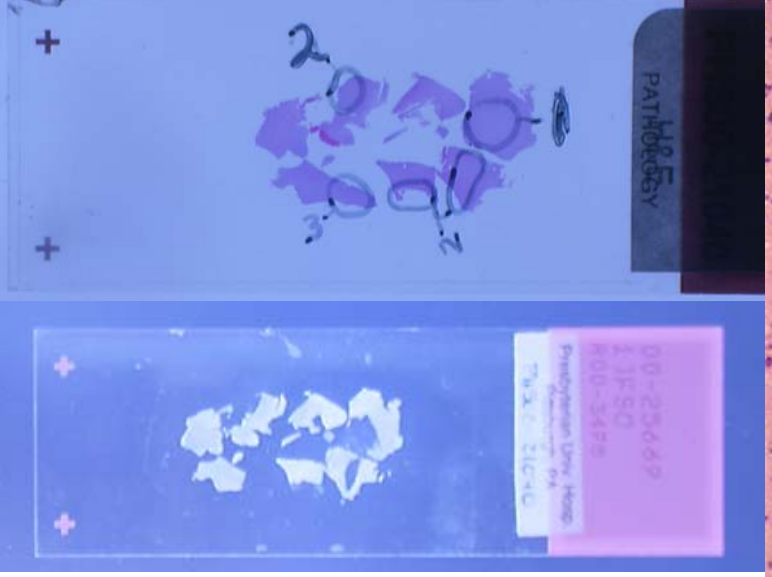
- PROGNOSTICATION
  - 1p deletion: high treatment response
  - Long term survivors: 1p deleted
  - High and low risk low grades
    - Mutational index: mutated/total informative
      - Fibrillary astrocytomas
      - Pilocytic astrocytomas
      - Pleomorphic xanthoastrocytomas

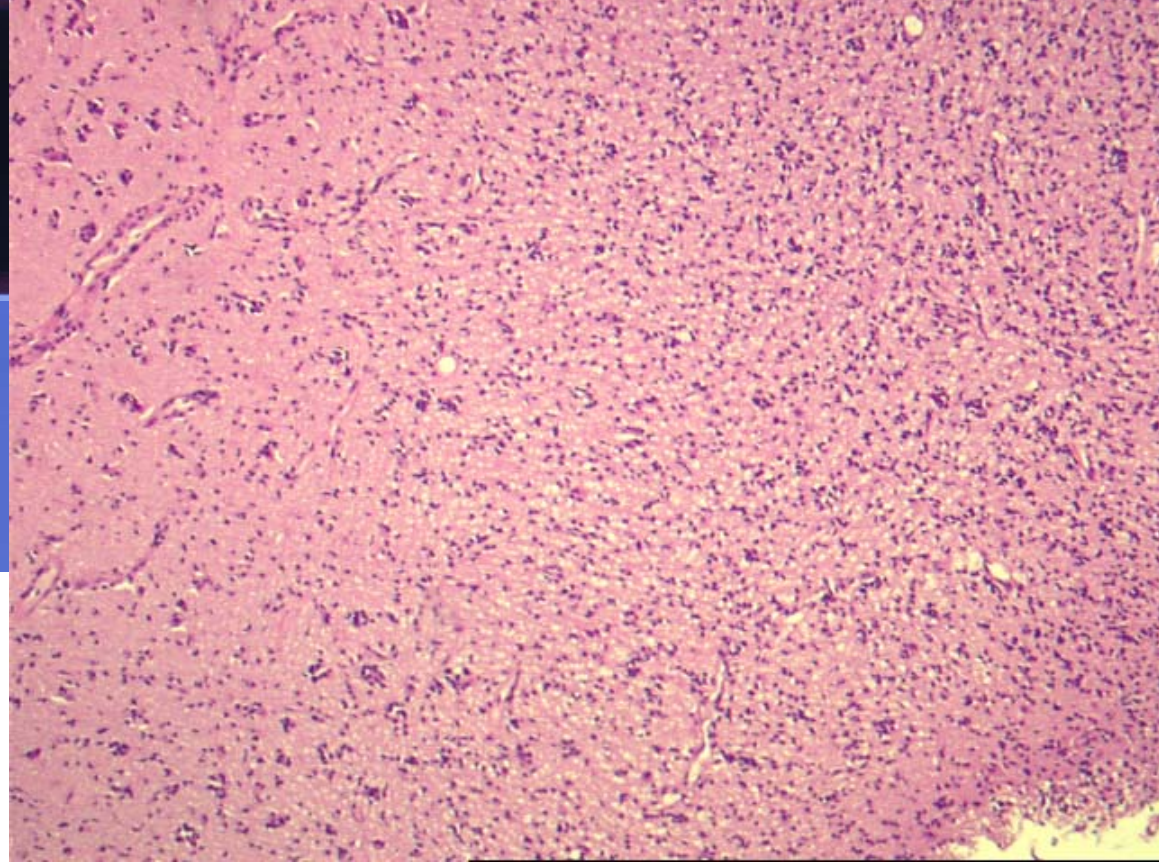
GLIOBLASTOMA MULTIFORME GENETIC PROFILING

- 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

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



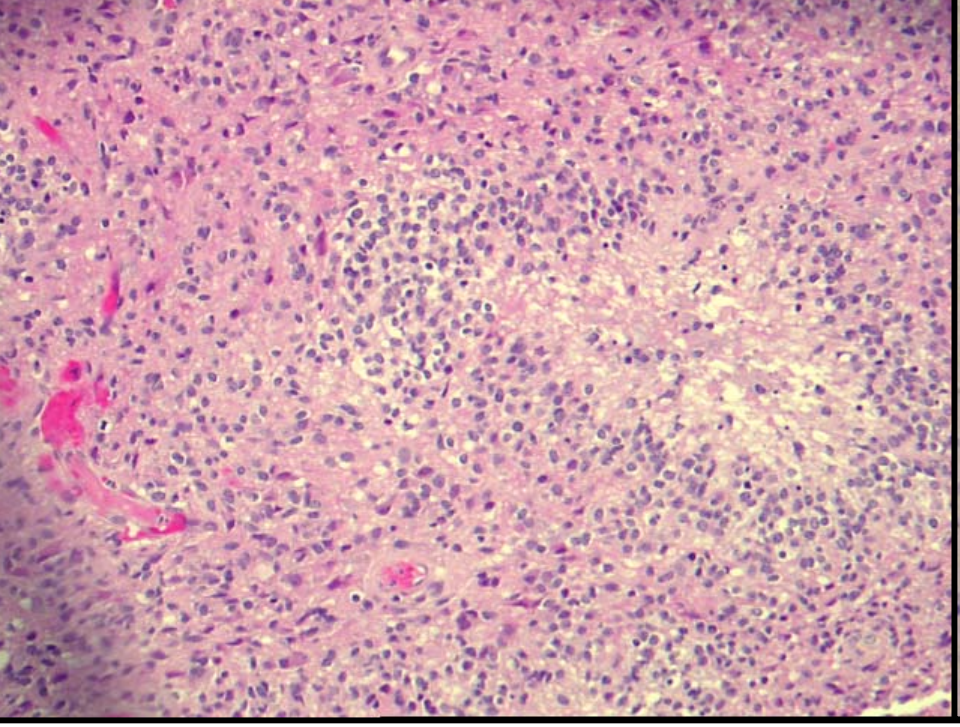


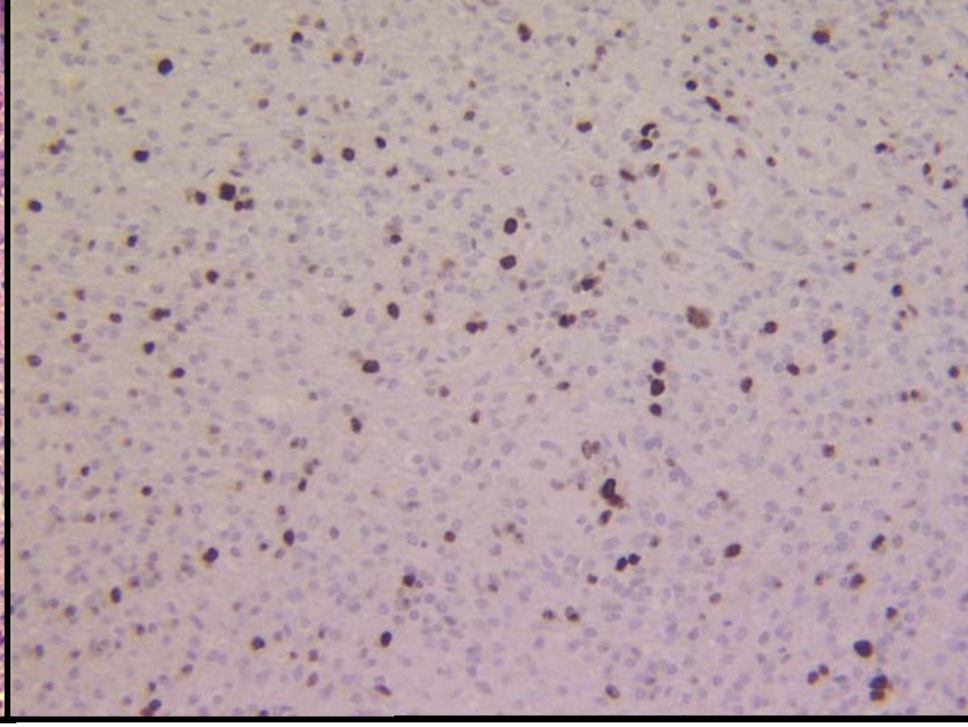
FRACTIONAL ALLELIC LOSS:

 # MUTATED MICROSATELLITES DIVIDED BY

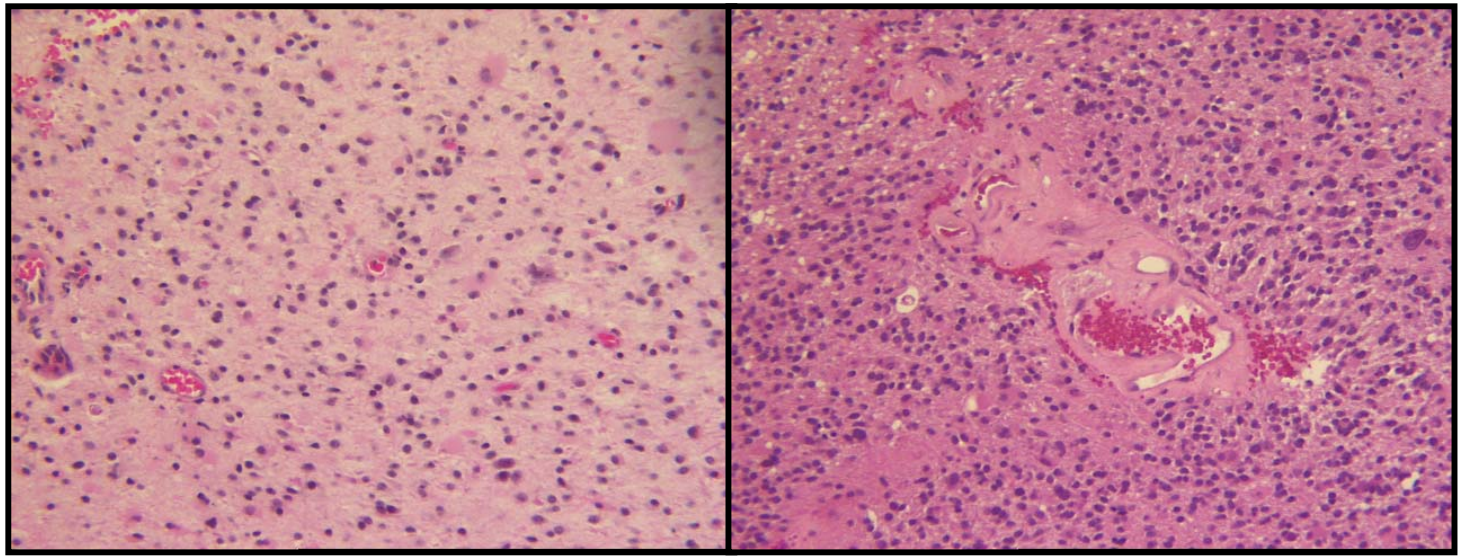
 # TOTAL INFORMATIVE MICROSATELLITES

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