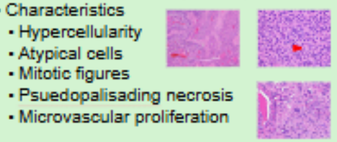
Analysis of BRAF V600E Mutation in a Cohort of Patients with Glioblastoma Using Tissue Microarrays

Introduction

Glioblastoma Multiforme

- WHO Grade IV astrocytic neoplasm of CNS
- Characteristics
  - Hypercellularity
  - Atypical cells
  - Mitotic figures
  - Psuedopalisading necrosis
  - Microvascular proliferation



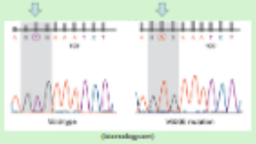
(AANS, 2015)

- Average survival is **14 months** (AANS, 2015)
- **Less than 3%** diagnosed survive 5 years
- Standard of care is ineffective
  - Surgical resection

  - Chemotherapy
  - Radiation

BRAF V600E point mutation

- 7-15% of all human cancers
  - Melanoma (Brandner et al., 2015)



Review of Literature

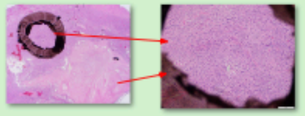
- BRAF V600E is present in 5% of high grade glioma, including glioblastoma (Schindler et al., 2011)
  - 10% pediatric high grade glioma
  - 50% of epithelioid (Gutmann et al., 2013)
- Identified using immunohistochemistry or sequencing
- Inhibitor PLX4032, or vemurafenib
  - FDA approved in 2011
  - High response rates in melanoma (Nikgoda et al., 2015)
    - Patients
    - Cell lines
- Applications in patients with glioblastoma
  - Robinson et al., 2014 and DeMasters et al., 2015

Purpose

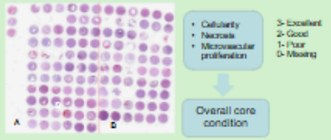
- Determine incidence of BRAF V600E in a cohort of glioblastoma using tissue microarrays
- Show that tissue microarrays are an effective screening method

Methodology

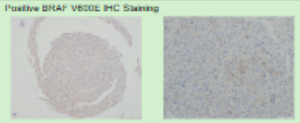
Create Two Tissue Microarrays



Analyze HE Stained Tissue Microarrays



Analyze BRAF IHC Tissue Microarrays



Confirm BRAF Positive Cases

Next Generation mutation sequencing

Results

Tissue Microarrays

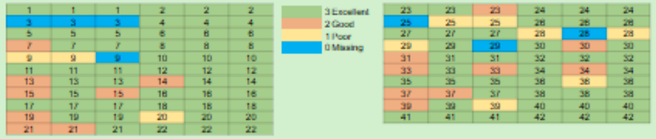
- Two microarrays created
- 40 and 42 cases
- 3 cores tissue per case

Characteristics of Cohort

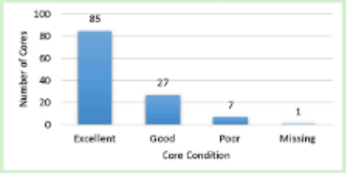
- Years 2010-2014
- Patients aged 9-86 years
  - 57.5 years average
- 37 female and 45 male

Overall Core Condition

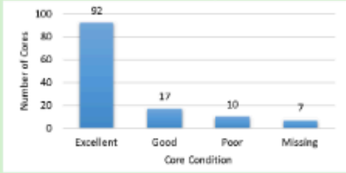
Tissue Microarray 1 Core Map



Overall Core Quality TMA 1

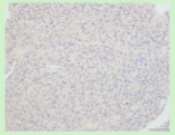


Overall Core Quality TMA 2



BRAF V600E Immunohistochemistry

- 4 cases positive by BRAF V600E IHC stained tissue microarray
  - Positive full slides
- 2 cases confirmed positive by next generation sequencing
  - Normal histological features



BRAF V600E IHC positive

Discussion

Tissue Microarray Quality

- Tissue Microarrays were of excellent quality
  - TMA 1 86.5% good and excellent
  - TMA 2 91% good and excellent
- Very few cores missing entirely
- Tissue microarrays are an effective method

Incidence of BRAF V600E Point Mutation

- 4/82 cases or 4.88% positive by IHC
- 2/82 cases or 2.45% confirmed positive by sequencing
  - Slightly lower than the common incidence
- Current BRAF V600E stain is 50% accurate at identifying positive cases

Conclusion

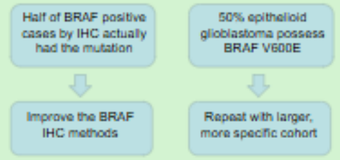
Tissue Microarrays	Whole Slides or Sequencing
Require **less resources**	More IHC stain required
Less costly	Very costly
Additional cuts of the array can be used to screen for additional mutations	Not ideal for screening every patient

All patients should be screened despite low incidence because of successful treatment

Significance

- **New treatment** for two patients identified
- **Better screening methods** increases the possibility of finding mutation and helping patients

Future Work



References