Tumor Mutational Burden as a Biomarker for Immunotherapy

David Fabrizio, VP, Translational Strategy, FMI

10 Dec 2020

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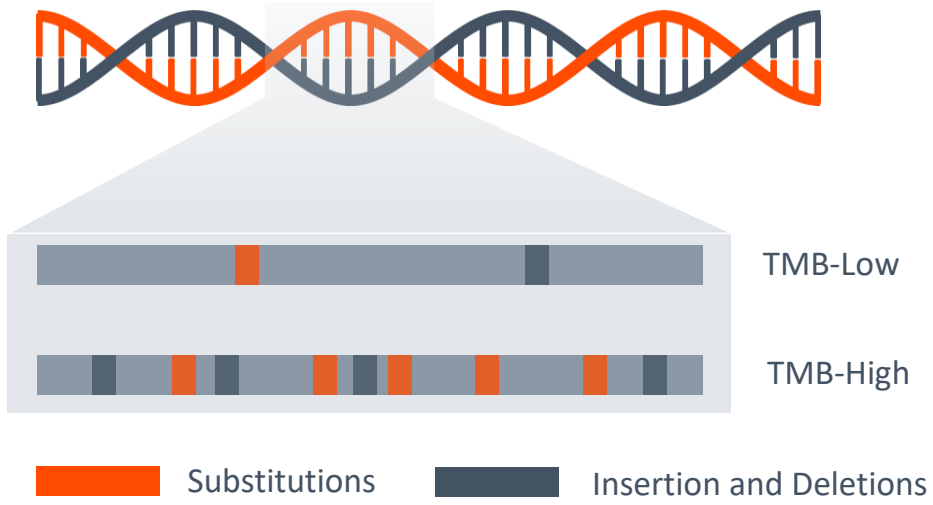
Defining Tumor Mutational Burden



Tumor mutational burden is an algorithmic measurement of a subset of synonymous and non-synonymous substitution and insertion/deletion mutations in a tumor's genome

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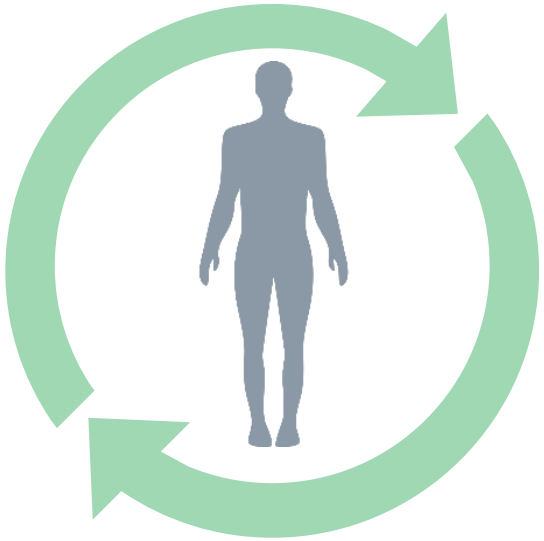
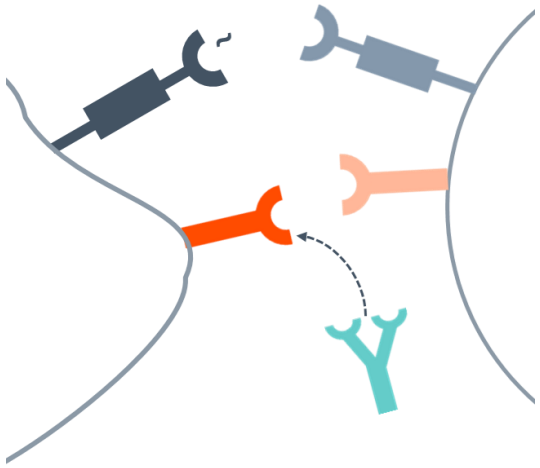
Tumor Mutational Burden Is Appropriate Across All Advanced Solid Tumors





TMB measurement is appropriate across all solid tumors, with all solid tumor companion diagnostic indications, inclusion in an increasing number of leading guidelines, and up to 15% of all advanced solid tumors reporting TMB-high

Data on File, Foundation Medicine, Inc., 2020
Yarchoan, Mark, et al. "PD-L1 Expression and Tumor Mutational Burden Are Independent Biomarkers in Most Cancers." JCI Insight, American Society for Clinical Investigation, 21 Mar. 2019, doi.org/10.1172/jci.insight.126908.
Chalmers, Z.R., Connelly, C.F., Fabrizio, D. *et al.* Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* **9,** 34 (2017). https://doi.org/10.1186/s13073-017-0424-2

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TMB is a Continuous Variable, But Limited to a Categorical Cutoff

- **Single cutoffs for continuous variable biomarkers presume the clinical response is evenly distributed within high and low cohorts**
- **Continuous variables distribute the clinical effect across a wider range of biomarker values**
  - For example, 11 and 30 mut/Mb are both considered TMB-H, but may have drastically different chances of clinical response

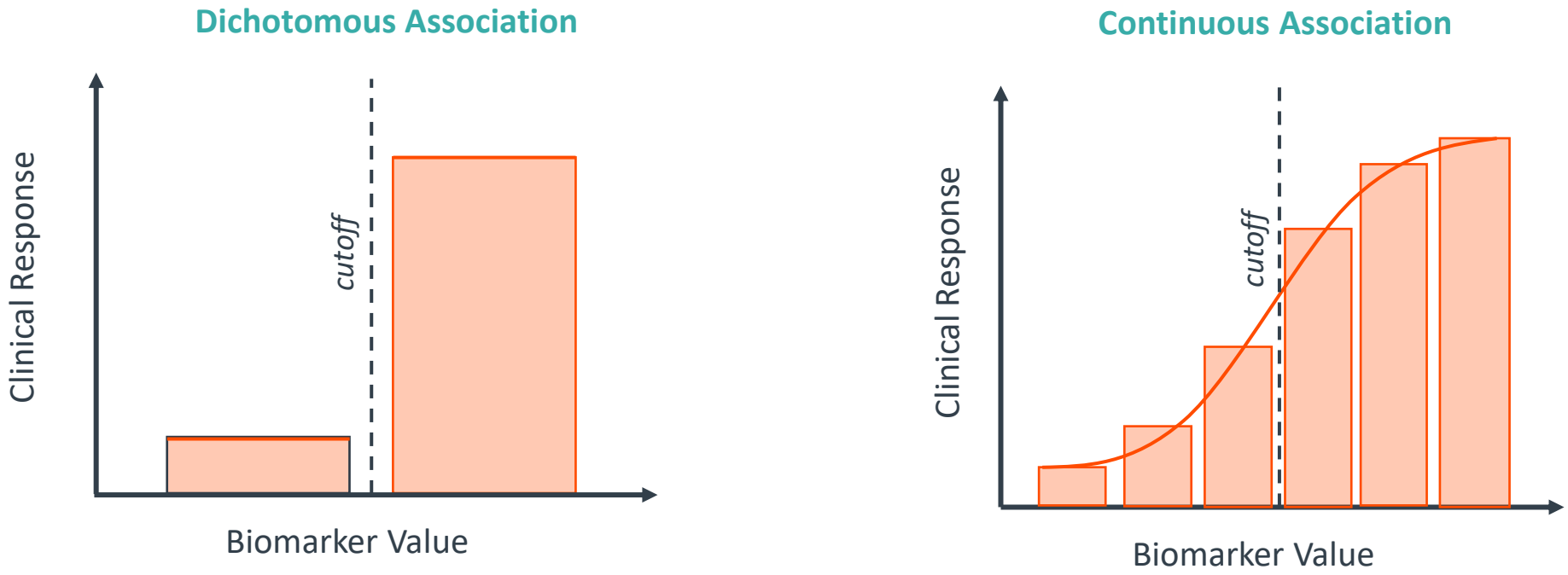

Figure adapted from Comment *et al.*, ***Clin Pharmacol Ther***, 2020

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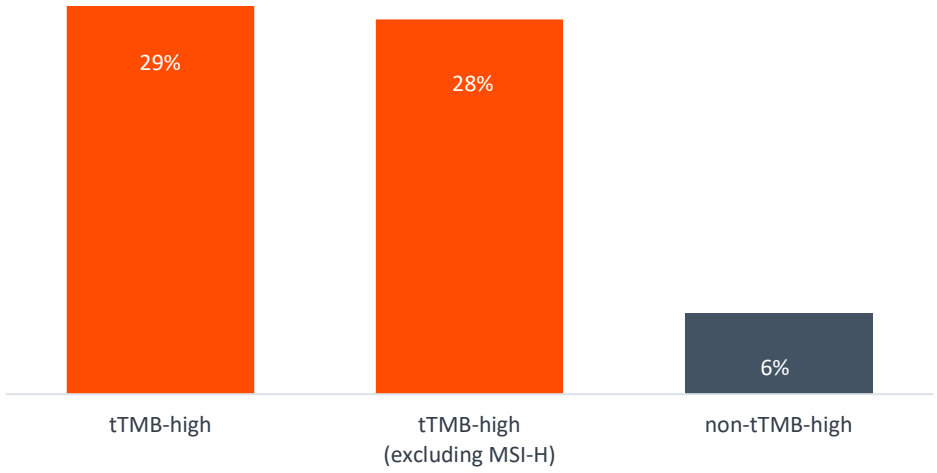
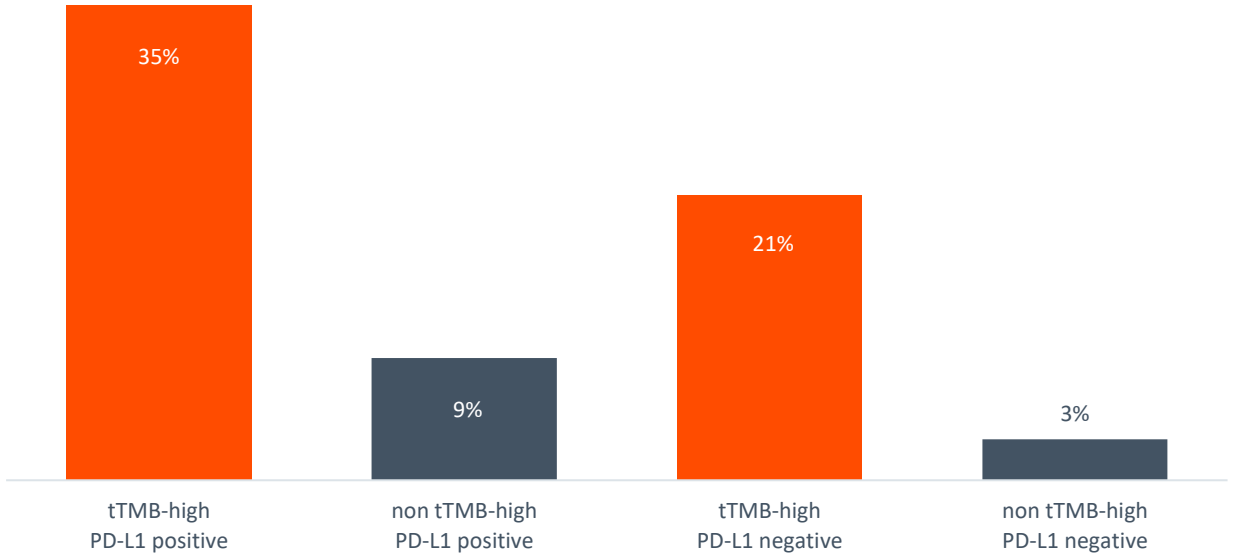
Tumor Mutational Burden as an Independent Predictive Biomarker

Overall Response Rate to Immunotherapy Based on TMB Status



Overall Response Rate to Immunotherapy Based on TMB and PD-L1 Status



Foundation Medicine. FoundationOne CDx Summary of Safety and Effectiveness Data. FDA. http://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S016B.pdf Accessed August 19, 2020

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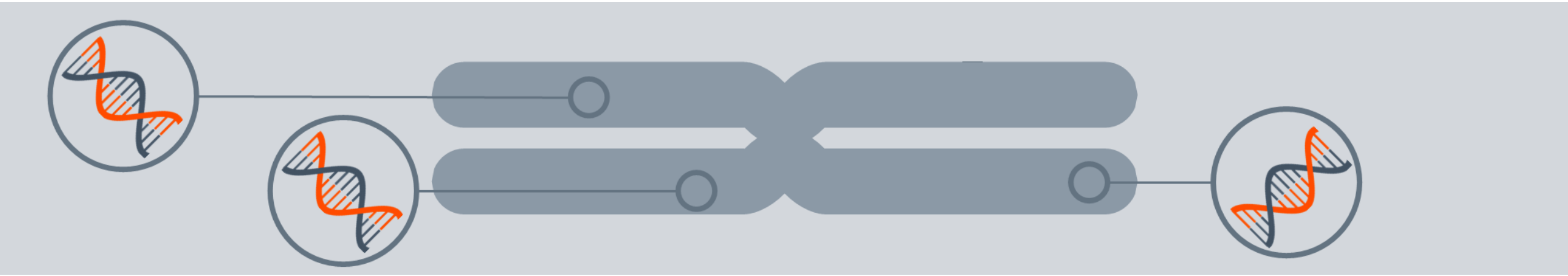
A Targeted Assay of Sufficient Size Is Needed to Calculate TMB



Whole exome sequencing was the initial method to calculate TMB, however it presents challenges in that it is **costly**, **time consuming**, and **not practical for clinical decision making**

Utilizing FoundationOne®CDx allows for the calculation of TMB in a cost- and time-effective manner, with **proven accuracy and clinical utility and is the only FDA-approved CDx with a TMB indication**

Merino, Diana M., et al. "Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project." Journal for Immunotherapy of Cancer 8.1 (2020).

FoundationOne®CDx is a qualitative next-generation sequencing based in vitro diagnostic test for advanced cancer patients with solid tumors and is for prescription use only. The test analyzes 324 genes as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) and is a companion diagnostic to identify patients who may benefit from treatment with specific therapies in accordance with the approved therapeutic product labeling. Additional genomic findings may be reported and are not prescriptive or conclusive for labeled use of any specific therapeutic product. Use of the test does not guarantee a patient will be matched to a treatment. A negative result does not rule out the presence of an alteration. Some patients may require a biopsy. For the complete label, including companion diagnostic indications and important risk information, please visit www.F1CDxLabel.com

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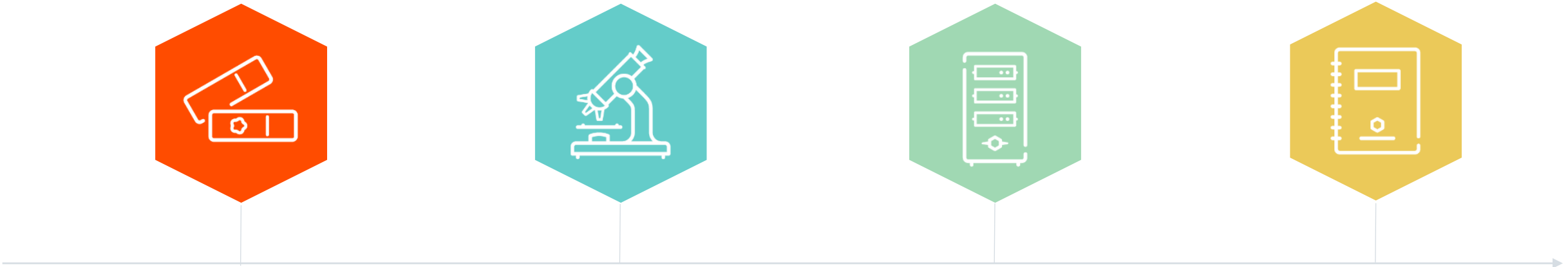
Why is Having a Validated TMB Assay Important?



Sample Collection and Processing

Sample Sequencing

Bioinformatic Processing

Reporting

Complexity in TMB calculation may lead to variation among different testing options

Stenzinger, A., Allen, J., Maas, J., Stewart, M., Merino, D., Wempe, M. and Dietel, M., 2019. Tumor mutational burden standardization initiatives: Recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. Genes, Chromosomes and Cancer, 58(8), pp.578-588.

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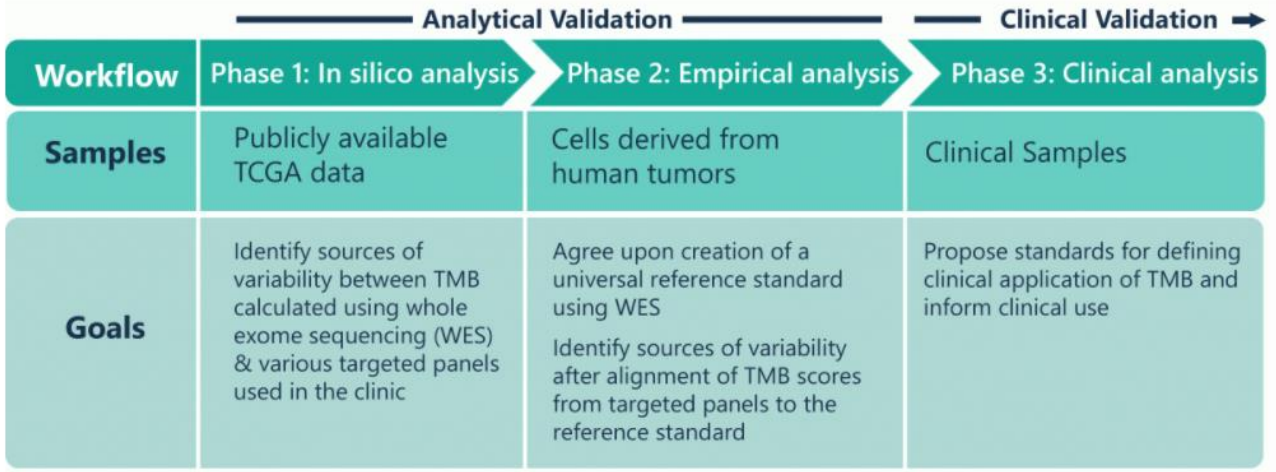
The TMB Harmonization Working Group Aims to Solve the Challenge of TMB Standardization



Government

- National Cancer Institute (NCI)
- U.S. Food and Drug Administration (FDA)

Academia

- Brigham & Women's Hospital
- College of American Pathologists
- Columbia University
- EORTC
- Genomic Testing Cooperative
- Hartwig Medical Foundation
- Johns Hopkins University
- Massachusetts General Hospital
- MD Anderson Cancer Center
- Memorial Sloan Kettering Cancer Center
- Quality in Pathology (QuIP)
- University of Heidelberg

Operational

- precisionFDA
- SeraCare

Diagnostics

- ACT Genomics
- Biodesix
- Caris Life Sciences
- Foundation Medicine, Inc.
- Guardant Health, Inc.
- Illumina, Inc.
- Intermountain Precision Genomics
- NeoGenomics Laboratories, Inc.
- OmniSeq
- Personal Genome Diagnostics (PGDx)
- Q2 Solutions
- QIAGEN, Inc.
- Quest Diagnostics
- RocheDx
- Thermo Fisher Scientific
- Thrive

Industry

- AstraZeneca
- Bristol-Myers Squibb Company
- EMD Serono, Inc.
- Genentech
- Merck & Co., Inc.
- Pfizer, Inc.
- Regeneron Pharmaceuticals

Friends of Cancer Research. 2020. Tumor Mutational Burden (TMB). [online] Available at: <https://www.focr.org/tmb> [Accessed 14 August 2020].

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In Silico Analysis of Theoretical TMB Variance Across Panels

TCGA dataset

Source: PanCanAtlas (MC3)
1 training set
1 validation set

TCGA WES

TCGA data down sampled to genes on panel

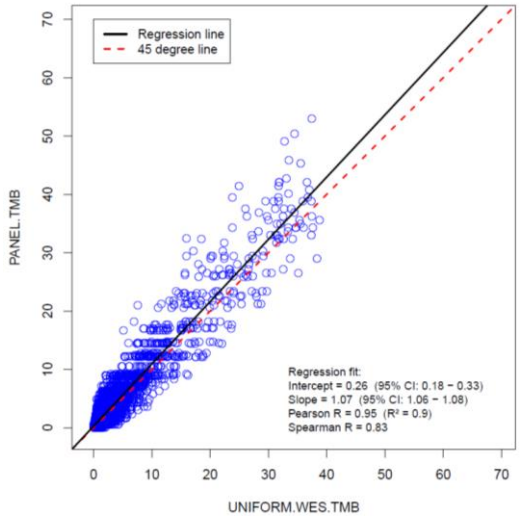
Consortium agreed TMB algorithm (Uniform Method)

Vendor TMB algorithm

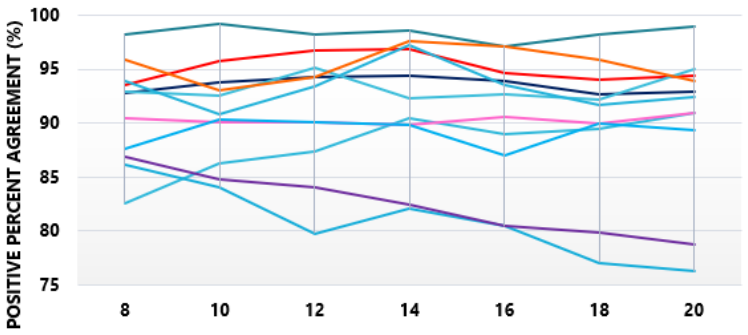
WES TMB Score

Panel TMB Score

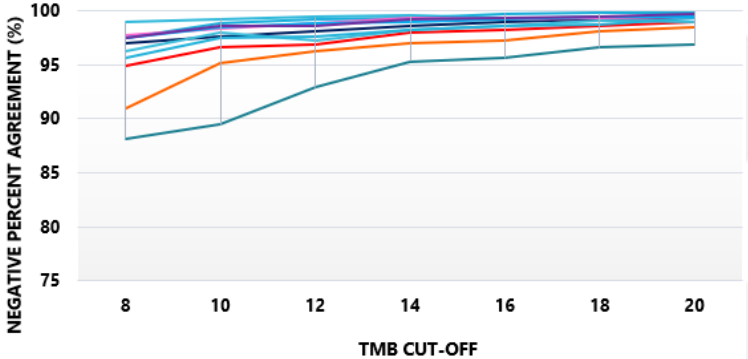
Concordance WES TMB vs. Panel TMB scores



Positive Percent Agreement for TMB cut-offs 8 - 20



Negative Percent Agreement for TMB cut-offs 8 - 20



Panel 1 Panel 2 Panel 3 Panel 4 Panel 5 Panel 6 Panel 7 Panel 8 Panel 9 Panel 10 Panel 11

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What Type of Data Did the FDA Review for the TMB Companion Diagnostic Indication?

- Analytic accuracy and concordance to WES
- Analytical sensitivity (i.e., LoD, LoB)
- Analytic specificity
- Precision and reproducibility
- Other laboratory studies*

Non-Clinical Lab Studies

The performance of FoundationOne CDx in detecting high TMB (TMB-H) as a qualitative pan tumor biomarker with respect to the 10 mutations per Mb cut-off is supported by data using a broad range of tumor specimens across all validation studies

- FoundationOne CDx analysis of TMB in Keynote-158
  - Objective Response Rates for TMB-High vs. TMB-Low

Primary Clinical Study

The clinical performance of FoundationOne CDx for detecting TMB-H in patients with solid tumors was demonstrated in a prospectively planned retrospective analysis of specimens from patients enrolled in the KEYNOTE-158 clinical study of pembrolizumab**

WES: Whole exome sequencing; LoD: Limit of detection; LoB: Limit of blank

*Also included: carryover/cross-contamination, reagent lot interchangeability, stability, general lab equipment and reagent evaluation, and guard banding/robustness

** Also included: study population demographics and baseline parameters and accountability of sPMA cohort

Foundation Medicine. FoundationOne CDx Summary of Safety and Effectiveness Data. FDA. http://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019S016B.pdf Accessed August 19, 2020

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How Many and What Types of Studies Support the Clinical Utility of TMB as a Predictive Biomarker?

Prospective trials reporting outcomes of therapy selection utilizing FMI TMB

45+
Studies

25+
Tumor Types

10,000+
Patients

Meta-analyses of outcomes including FMI and other TMB Assays

Retrospective analyses reporting outcomes associated with FMI TMB

Published Studies on File, Foundation Medicine, Inc., 2020

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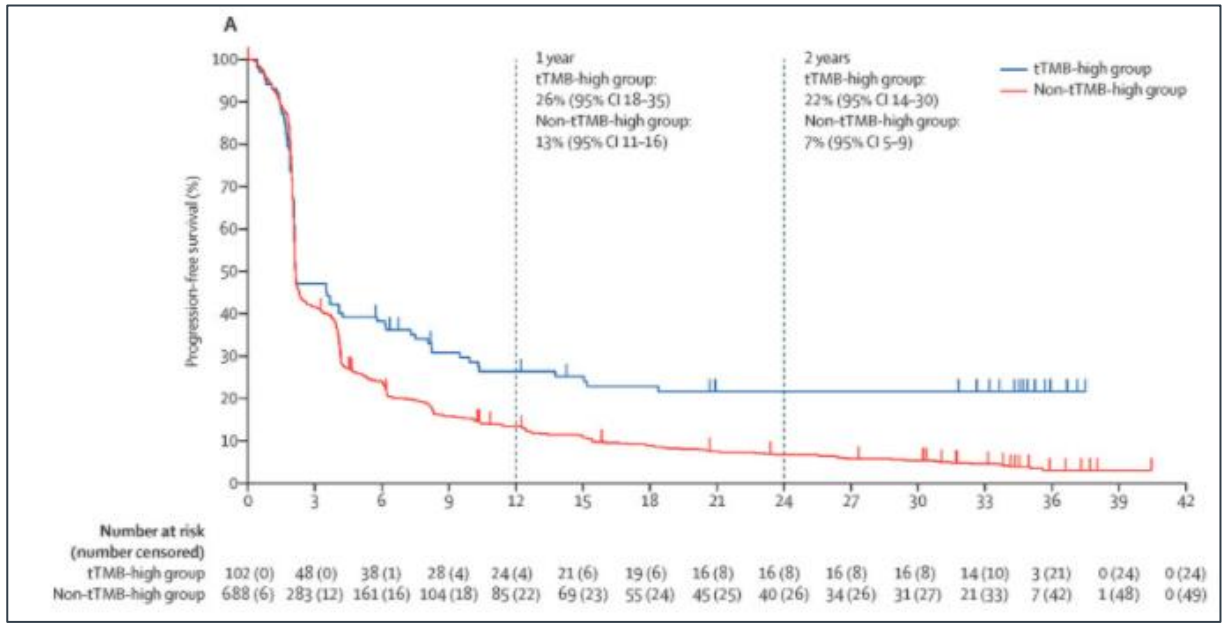
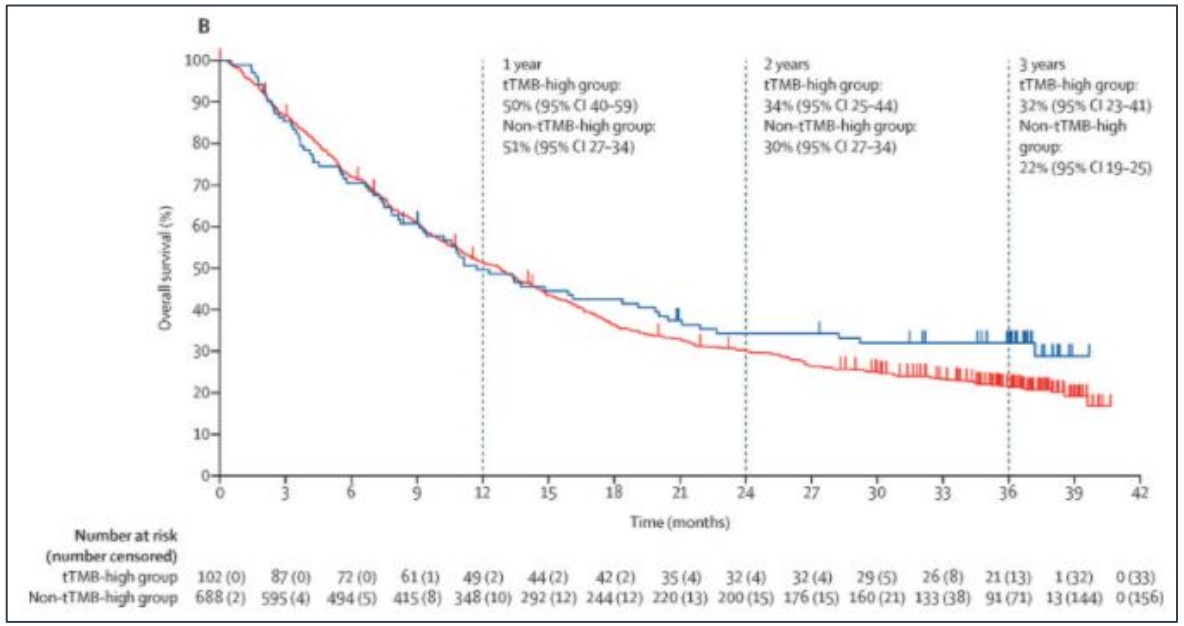
High TMB Associated with Increased PFS and OS

Keynote 158 Study evaluated 10 different tumor types

TMB Associated PFS in KN-158



TMB Associated OS in KN-158



Marabelle et al, Lanc Onc, 2020

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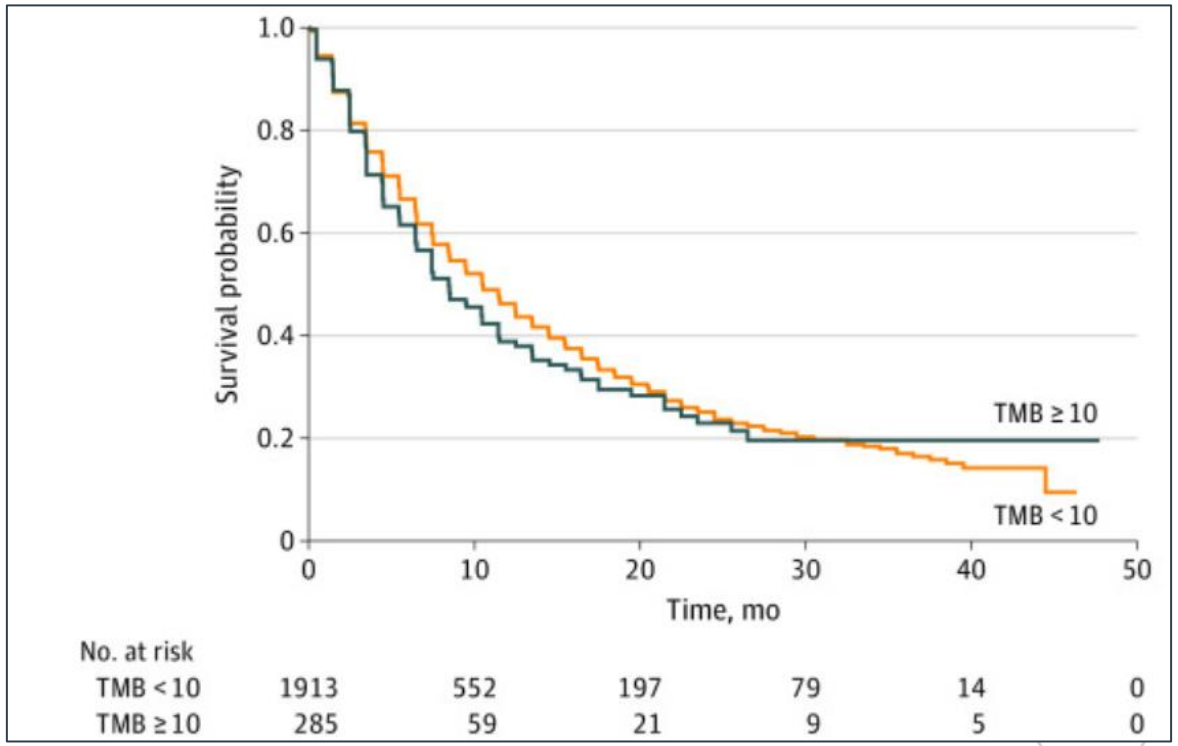
TMB Is Not Prognostic in Non-IO Treated Patients

No enrichment in survival observed using real-world data

TMB Associated OS in Non-IO Treated Patients



Shao et al, JAMA Net Open, 2020

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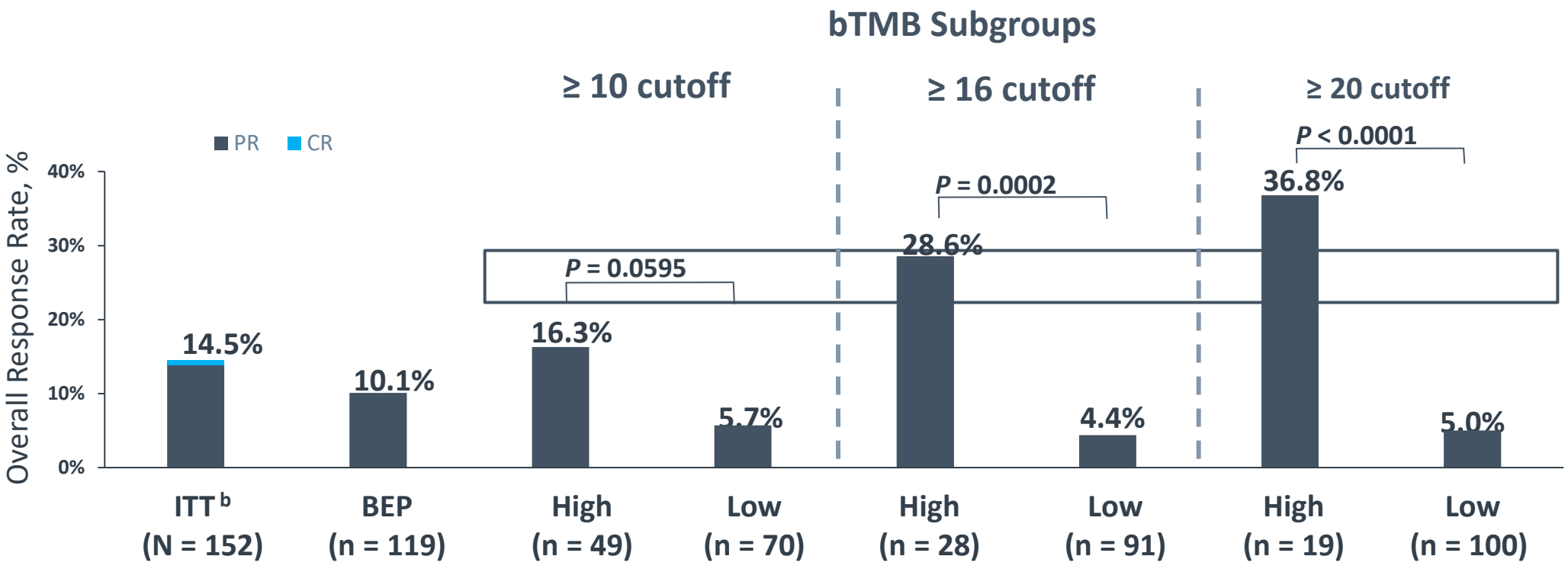
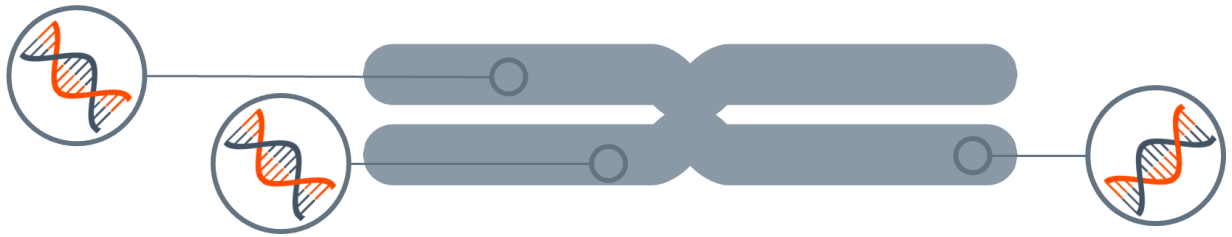
Blood-Based TMB is Associated with Enriched Response Rate

- Data presented by Kim et al. at ESMO 2018 in 1L NSCLC trial, B-FIRST, is first prospective demonstration that bTMB predicts response rate for atezo monotherapy



Kim et al. BF1RST Primary Analysis

Conclusions and Future Directions



- Clinical literature demonstrating clinical utility
- Friends of Cancer Research harmonization efforts
- FDA Approval as Pan-Tumor CDx
- Blood-based TMB emerging biomarker
- Continuous variable analysis should be considered in future studies

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ONKOMEDEOR
ONKOLOGISCHE ZENTREN

Prof. Dirk Hempel

Head of the Steinbeis Transfer Institute for clinical hemato-oncology at the Steinbeis University Berlin

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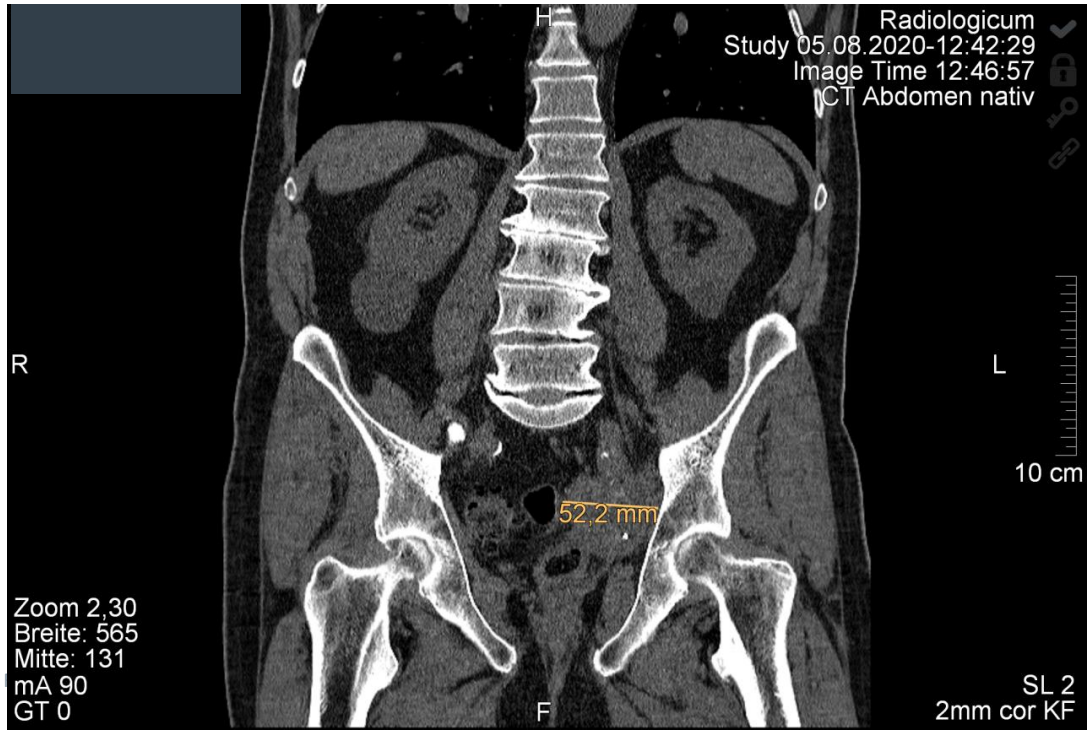
Primary Urothelial Carcinoma of Bladder

Case History

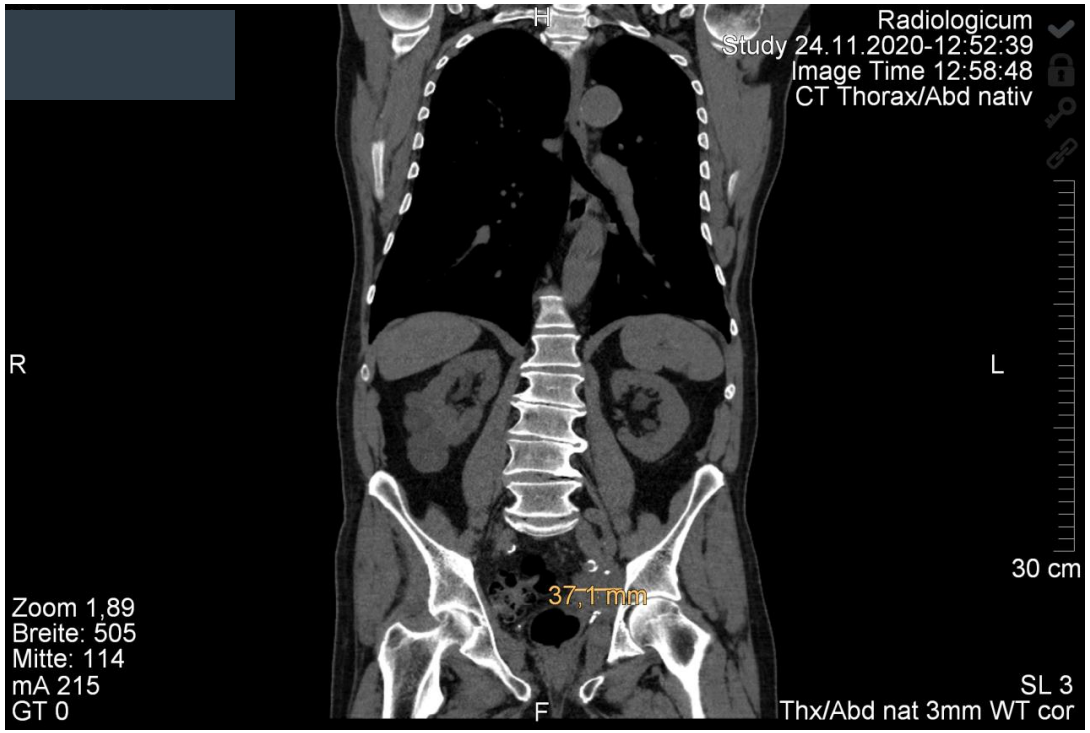
- 80-years-old-gentelman
- **August 2009**: K/c/o Urothelial cancer of bladder, pT2a, pN0, M0, high grade, cystectomy with ilium conduit
- Family history: no history of malignancies
- **April 2017: TFI 7.5 years**: Mesenteric and pararectal LN metastases --> Gemcitabine/Cisplatin 6 cycles -> complete remission was achieved
- **August 2020:** swelling of left leg was noticed
- Staging evaluation with abdominal CT: LN with a diameter of 5,2cm at left iliac vein
- Histology: Pelvic LN metastases consistent with urothelial cancer (CK7+, CK20+)
- **Since September 2020**: Pembrolizumab

ONKOMEDEOR
ONKOLOGISCHE ZENTREN

Progressive LN metastases pelvis left from 2,1cm (01/20) to 5,2cm 08/20



LN metastases left pelvis 3,7cm cm 11/20



Genomic Signatures

Tumor Mutational Burden - 19 Muts/Mb

Microsatellite status - MS-Stable

Gene Alterations

For a complete list of the genes assayed, please refer to the Appendix.

CD274 (PD-L1) amplification

PDCD1LG2 (PD-L2) amplification

PIK3CA E545K

CREBBP splice site 2463+1G>A

DNMT3A R771*

JAK2 amplification

NOTCH3 S945*

RBM10 splice site 2668-1G>A

TERT promoter -124C>T

9 Therapies approved in the EU

0 Therapies with Lack of Response

20 Clinical Trials

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Your opinion matters

POLL QUESTION #1

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Your opinion matters

POLL QUESTION #2

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ONKOLOGISCHE ZENTREN

Multigensequenzierung

SIGNATURES

Tumor Mutational Burden - 19 Muts/Mb--> Pembrolizumab, Atezolizumab, Nivolumab

MS-Stable

CD274 (PD-L1) - amplification--> Pembrolizumab, Atezolizumab, Nivolumab

PDCD1LG2 (PD-L2) - amplification--> --> Pembrolizumab, Atezolizumab, Nivolumab

PIK3CA - E545K---> Alpelisib, Everolimus, Temsirolimus

Current Treatment:

3. Zyklus Pembrolizumab 200 mg mono 06.11.2020 qd22 simultaneously with RT

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Biopsy from LN Metastases

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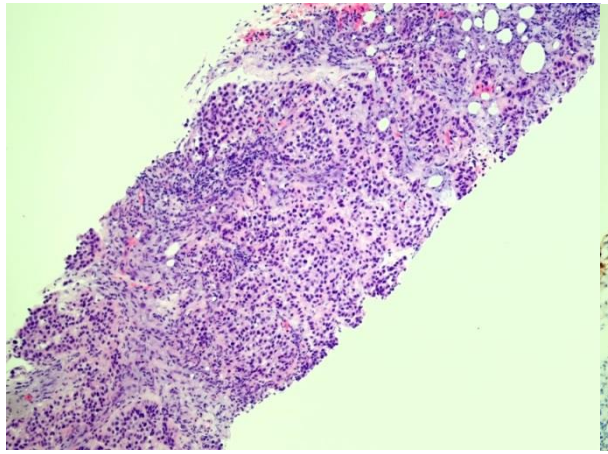
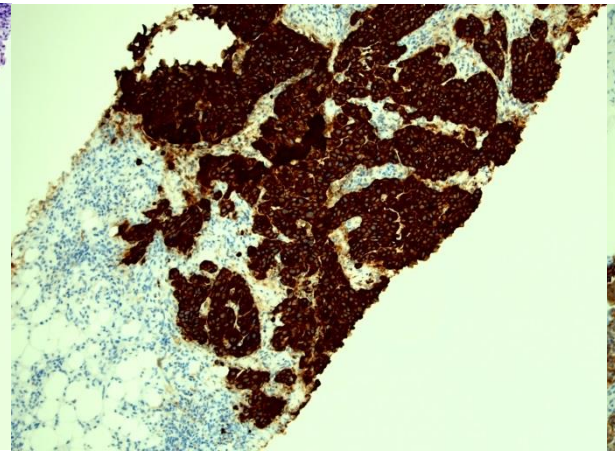
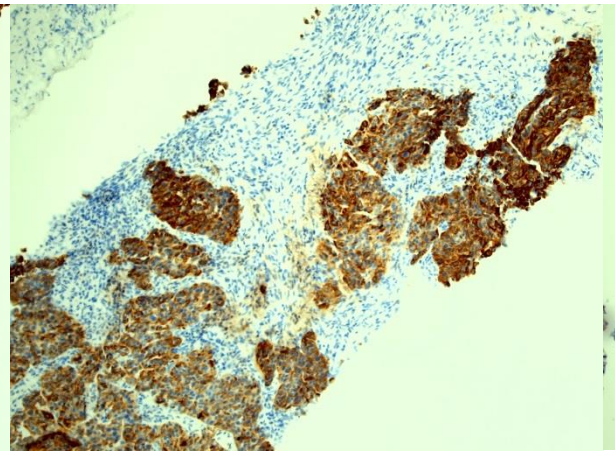


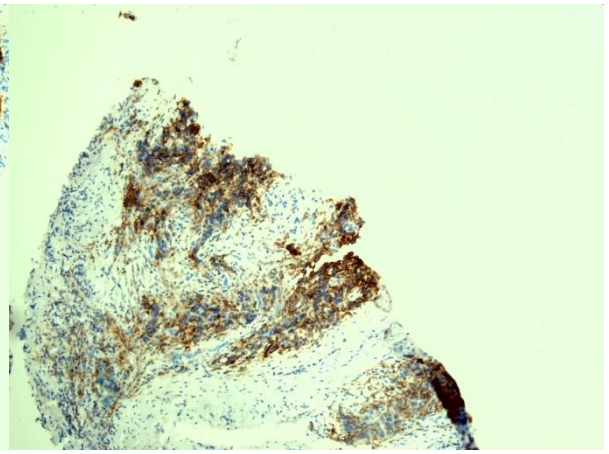
HE Staining



CK7 Staining



CK20 Staining



PDL1 Staining

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Mahidol University
Faculty of Medicine Siriraj Hospital

Molecular Tumor Board
Webinar Series

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Roche

A 65-year-old man with glioblastoma with high tumor mutational burden

Sith Sathornsumetee, MD

Department of Medicine (Neurology)

Faculty of Medicine Siriraj Hospital, Mahidol University

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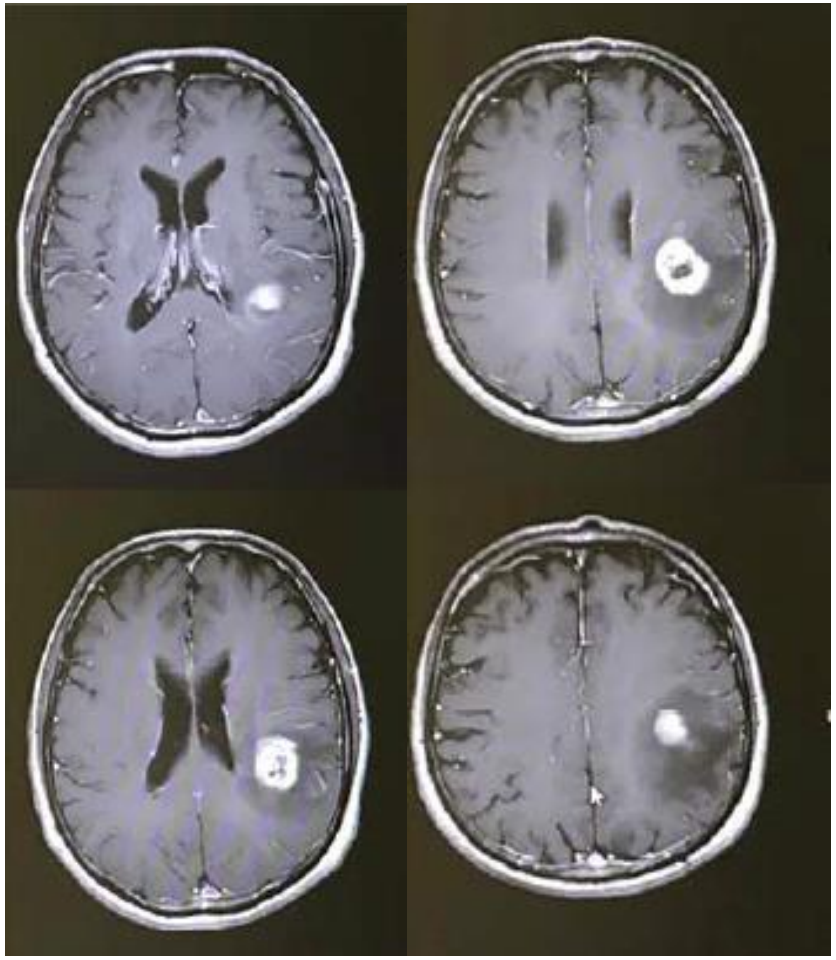
sith.sat@mahidol.edu

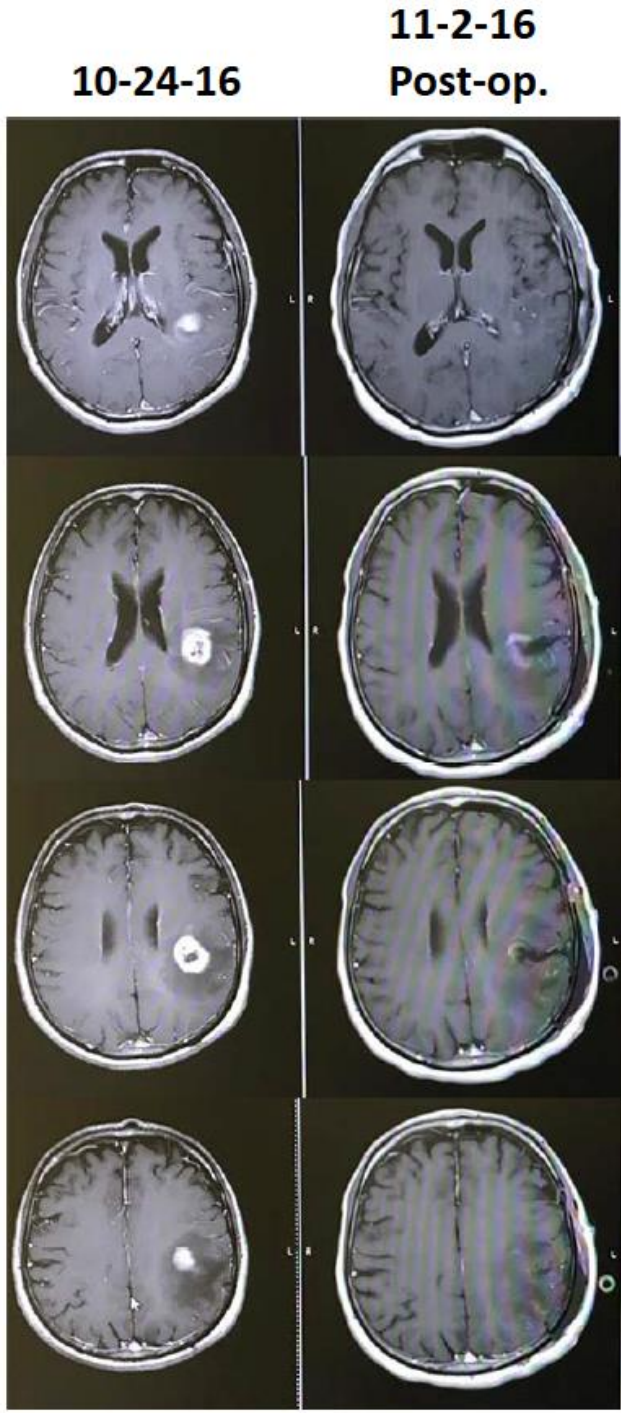
History

- A 65-year-old, previously healthy, Thai man presented in 10/2016 with naming difficulty and cognitive dysfunction
- Brain MRI (10/2016) revealed left frontoparietal nodular-ring enhancing lesion with surrounding vasogenic edema
- Awake craniotomy with gross total resection on 11/2016



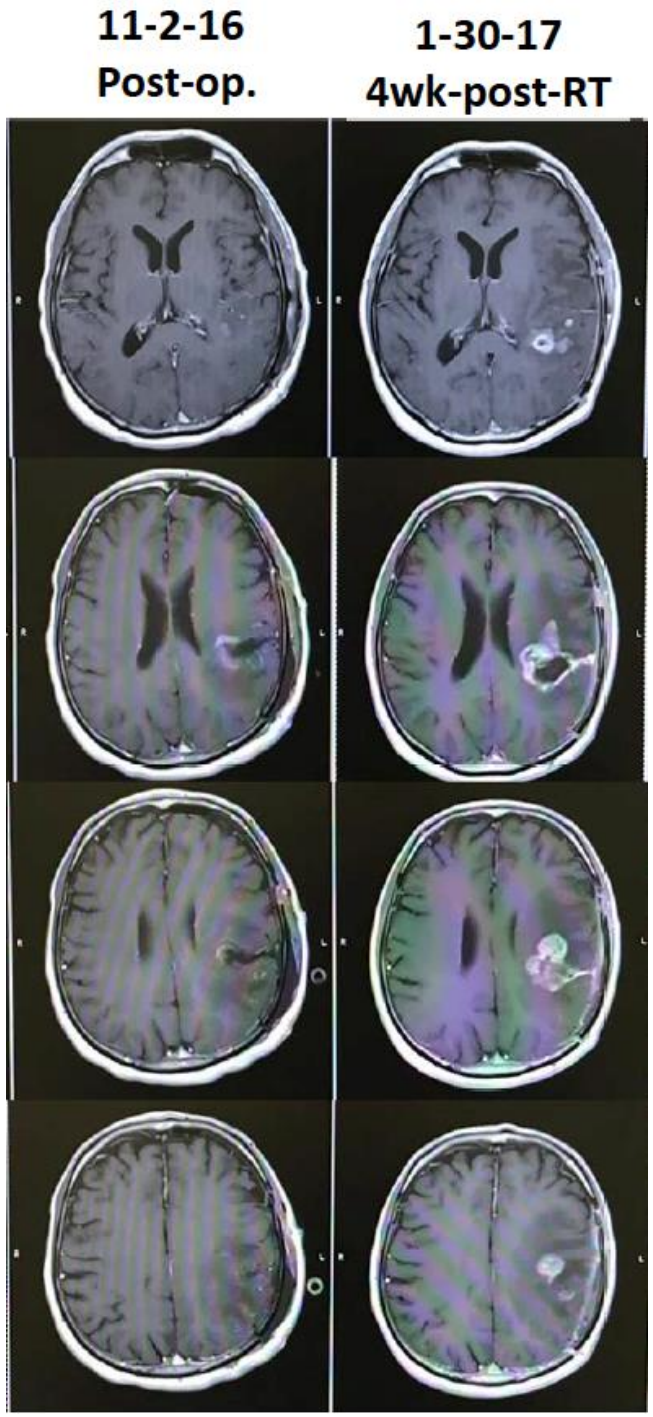
Clinical Presentation

- Post-operative MRI (11/2016): confirmed gross total resection of enhancing tumor
- Pathology: Glioblastoma (WHO grade IV), *IDH* wild-type, unmethylated *MGMT*
- KPS 80% with right UE weakness and motor aphasia
- Underwent conformal 3D-RT 60Gy with concurrent temozolomide (TMZ) (11/16-1/17)



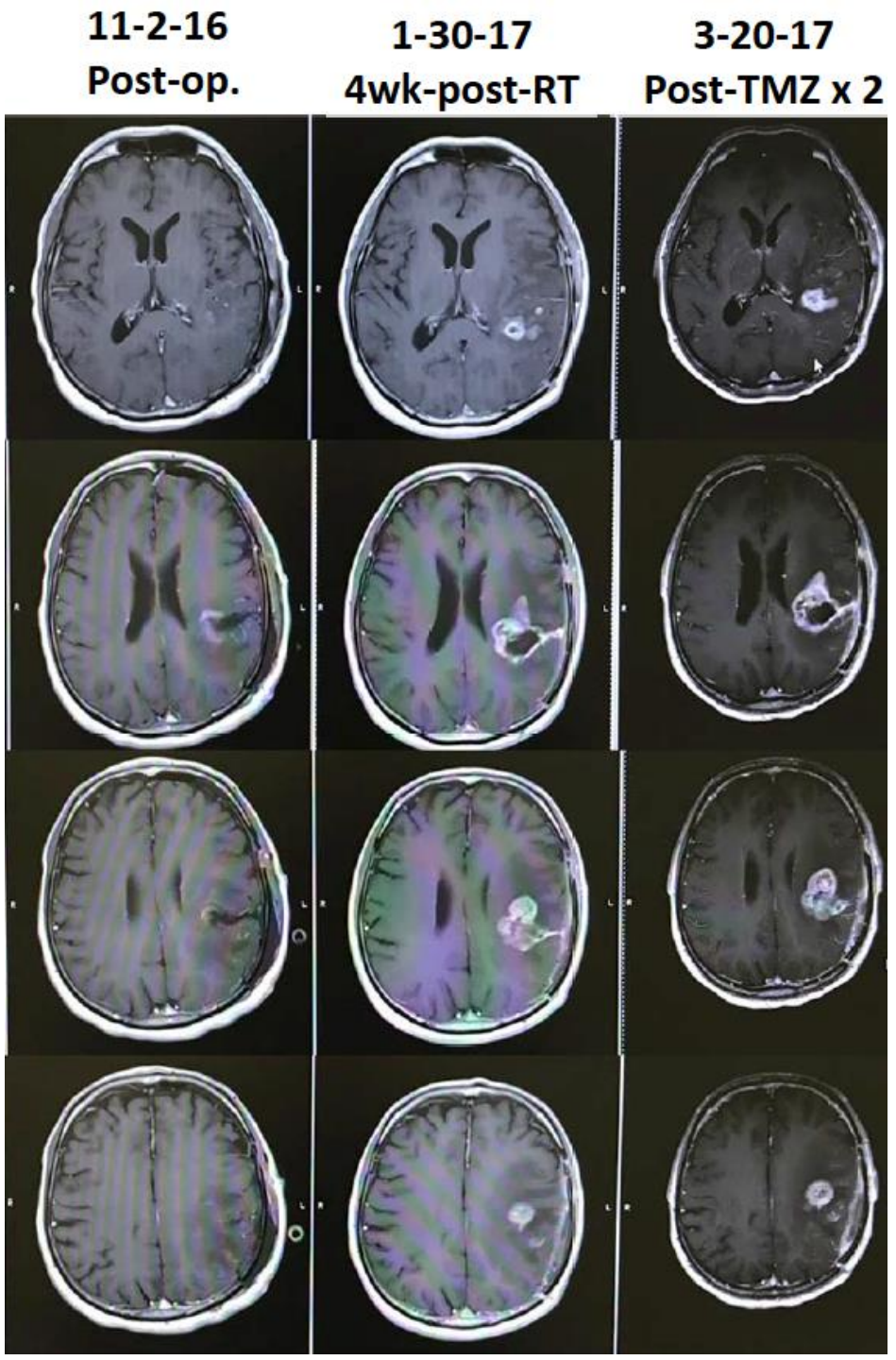
Clinical Presentation

- Brain MRI (1/17; 4 weeks after RT completion): increased nodular-ring enhancement with surrounding edema
  - Differential diagnosis:
    - True progression vs. pseudoprogression
- Adjuvant TMZ x 2 cycles and repeat brain MRI



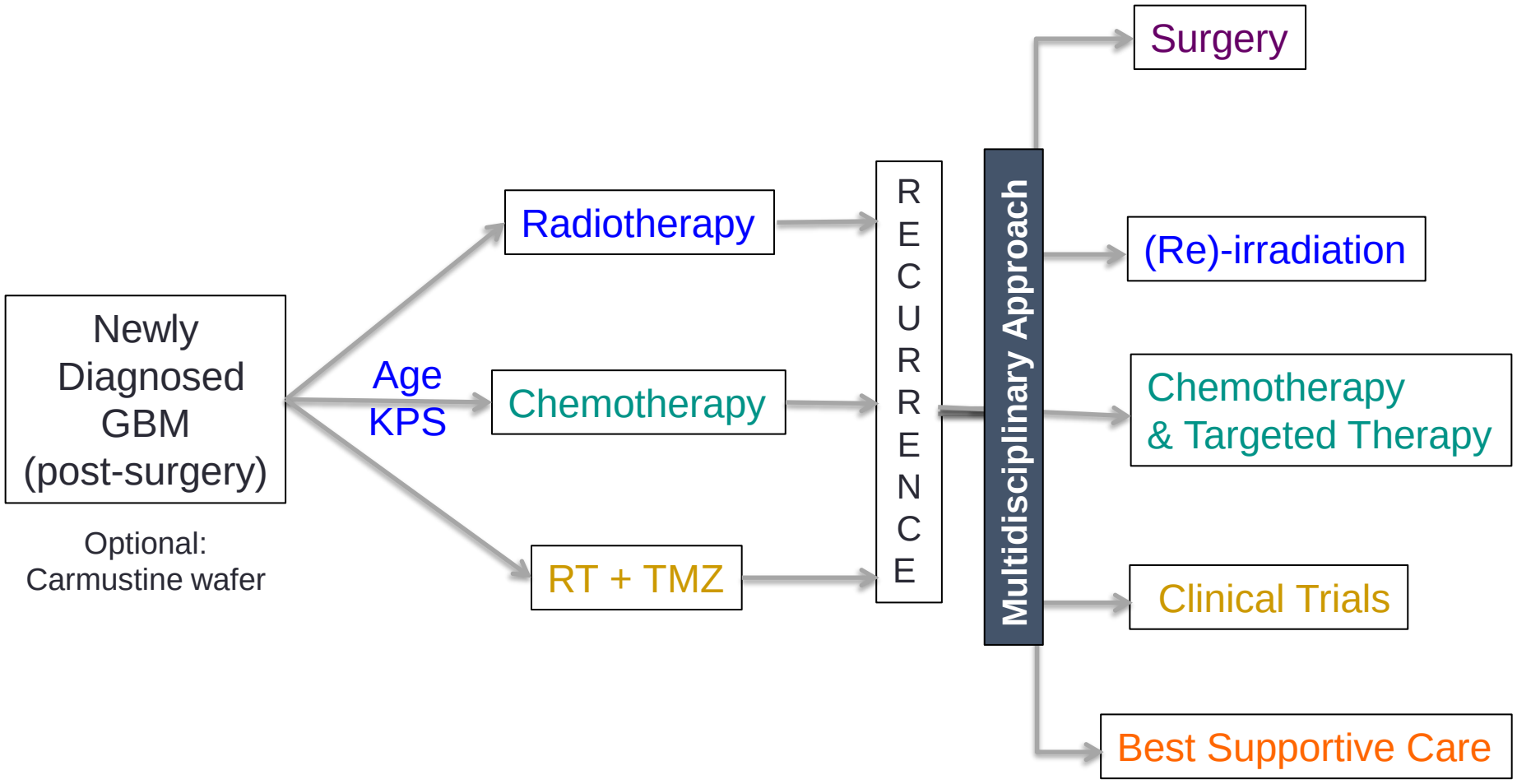
Clinical Presentation

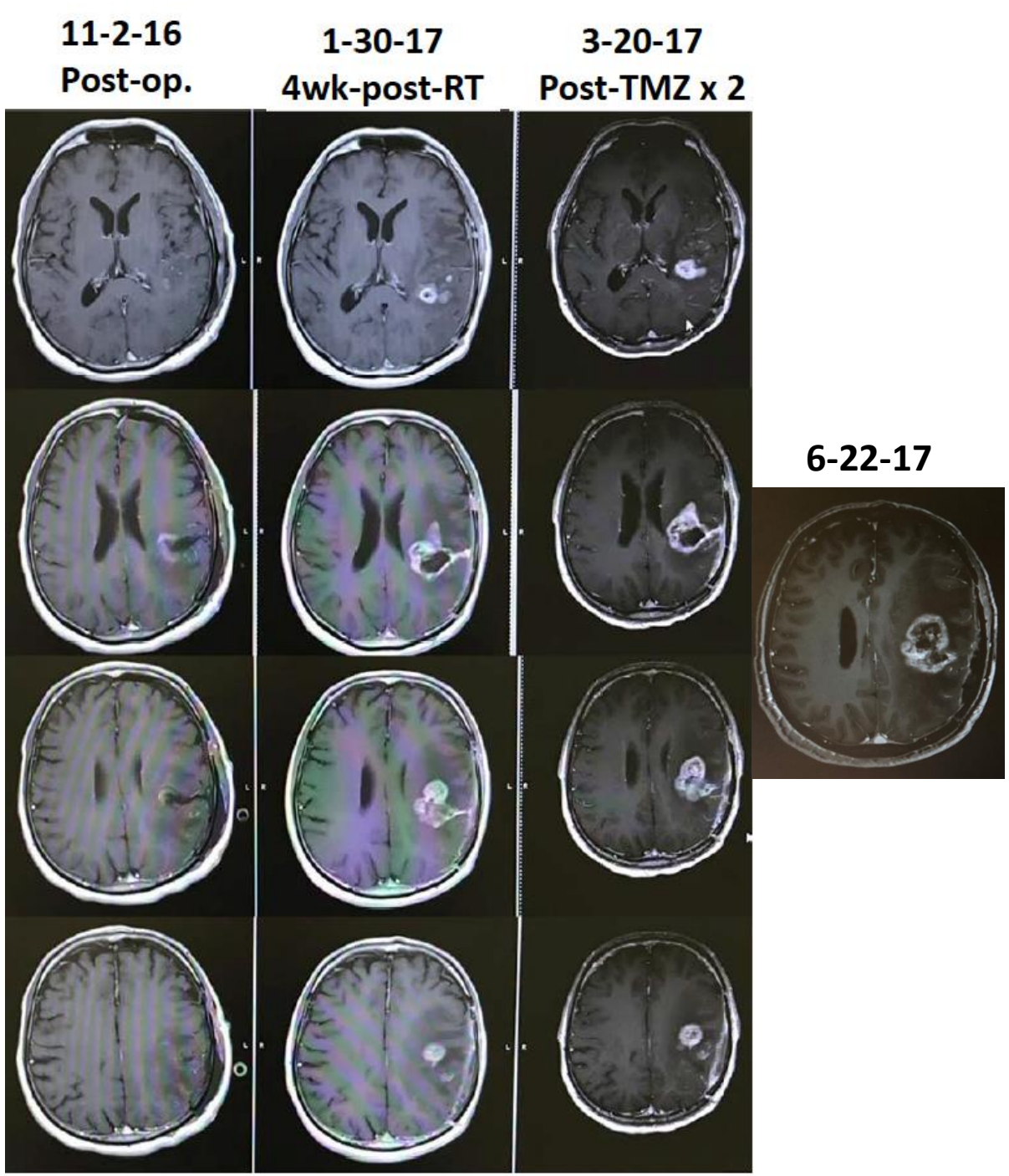
- Brain MRI (3/20/17): mild increase in size of enhancing lesion
- Clinical progression with more speech difficulty and right sided weakness
- Likely a true progression



Recurrent Glioblastoma

Treatment Options





Repeat Resection 7-17

Pathology: Glioblastoma with therapeutic effect

FoundationOne CDx

FoundationOne Report

18 August 2017

TUMOR TYPE: BRAIN GLIOBLASTOMA (GBM)

Genomic Alterations Identified†

NF1 E524*
PIK3CA G118D
PTEN D24Y, E299*
CBL splice site 1096-1G>T
CHD2 E1542*
CSF1R F971fs*7
PIK3R1 R348*
POLE P286R
ROS1 K1163N – subclonal#
SETD2 E463*, E581*
TP53 P151S, R213*

Additional Findings†

Microsatellite status MS-Stable

Tumor Mutation Burden TMB-High; 32 Muts/Mb

Additional Disease-relevant Genes with No Reportable Alterations Identified†

EGFR
IDH1
PDGFRA

VARIANTS OF UNKNOWN SIGNIFICANCE

Note: One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

APC A1402V	***ARID2*** S319Y	***BRAF*** I379V	***CD274*** Y208C	***CHD4*** D1328Y	***ERBB4*** S535Y
FLT4 S1163L	***GNAS*** A424V	***INPP4B*** R348I	***LZTR1*** D654A	***MLL3*** N3372S	***MTOR*** R2018*
PDGFRA R1039K	***PLCG2*** K535N	***POLE*** R1556W	***PREX2*** splice site 4088-1G>T	***SETD2*** E498D	***SF3B1*** S1150L
SPEN D1808V	***TSHR*** A471T				

PIK3R1 Protein Effect:R348* Mutation Allele Frequency (MAF)= 16.8%
TP53 Protein Effect:R213* Mutation Allele Frequency (MAF)= 18.5%
CBL Protein Effect:splice site 1096-1G>T Mutation Allele Frequency (MAF)= 12.3%
CSF1R Protein Effect:F971fs*7 Mutation Allele Frequency (MAF)= 47.9%
SETD2 Protein Effect:E463* Mutation Allele Frequency (MAF)= 18.0%
PTEN Protein Effect:E299* Mutation Allele Frequency (MAF)= 22.5%
ROS1 Protein Effect:K1163N Mutation Allele Frequency (MAF)= 1.7%
POLE Protein Effect:P286R Mutation Allele Frequency (MAF)= 17.4%
TP53 Protein Effect:P151S Mutation Allele Frequency (MAF)= 19.1%
PIK3CA Protein Effect:G118D Mutation Allele Frequency (MAF)= 10.4%
PTEN Protein Effect:D24Y Mutation Allele Frequency (MAF)= 2.0%
NF1 Protein Effect:E524* Mutation Allele Frequency (MAF)= 3.6%
CHD2 Protein Effect:E1542* Mutation Allele Frequency (MAF)= 26.6%
SETD2 Protein Effect:E581* Mutation Allele Frequency (MAF)= 15.4%

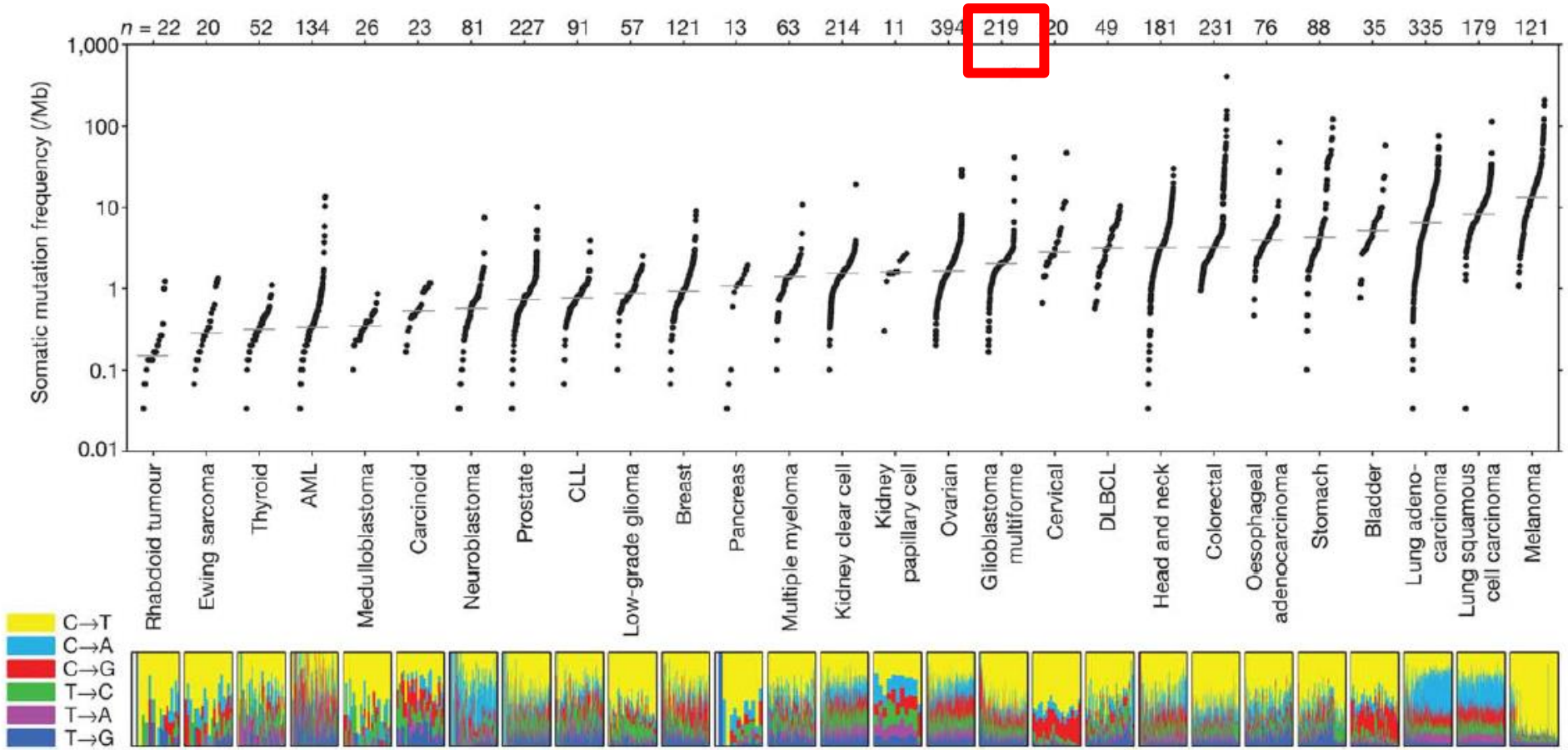
Genomic Findings Detected	FDA-Approved Therapies (in patient's tumor type)	FDA-Approved Therapies (in another tumor type)	Potential Clinical Trials
PIK3CA G118D	None	Everolimus Temsirolimus	Yes, see clinical trials section
PTEN D24Y, E299*	None	Everolimus Temsirolimus	Yes, see clinical trials section
Tumor Mutation Burden TMB-High; 32 Muts/Mb	None	Atezolizumab Avelumab Durvalumab Nivolumab Pembrolizumab	Yes, see clinical trials section
CBL splice site 1096-1G>T	None	None	None
CHD2 E1542*	None	None	None
CSF1R F971fs*7	None	None	None
Microsatellite status MS-Stable	None	None	None
PIK3R1 R348*	None	None	None
POLE P286R	None	None	None
ROS1 K1163N - subclonal	None	None	None
SETD2 E463*, E581*	None	None	None
TP53 P151S, R213*	None	None	None

Your opinion matters

POLL QUESTION #1

FOUNDATION MEDICINE® | Roche

Mutational Burden in Cancers



Lawrence et al. Nature 2013.

Article

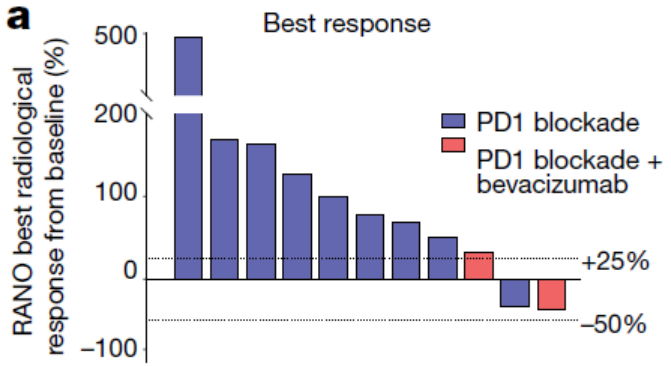
Mechanisms and therapeutic implications of hypermutation in gliomas

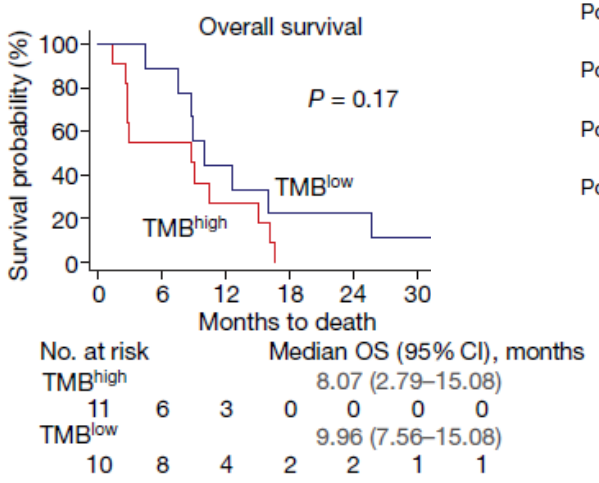
Nature | Vol 580 | 23 April 2020

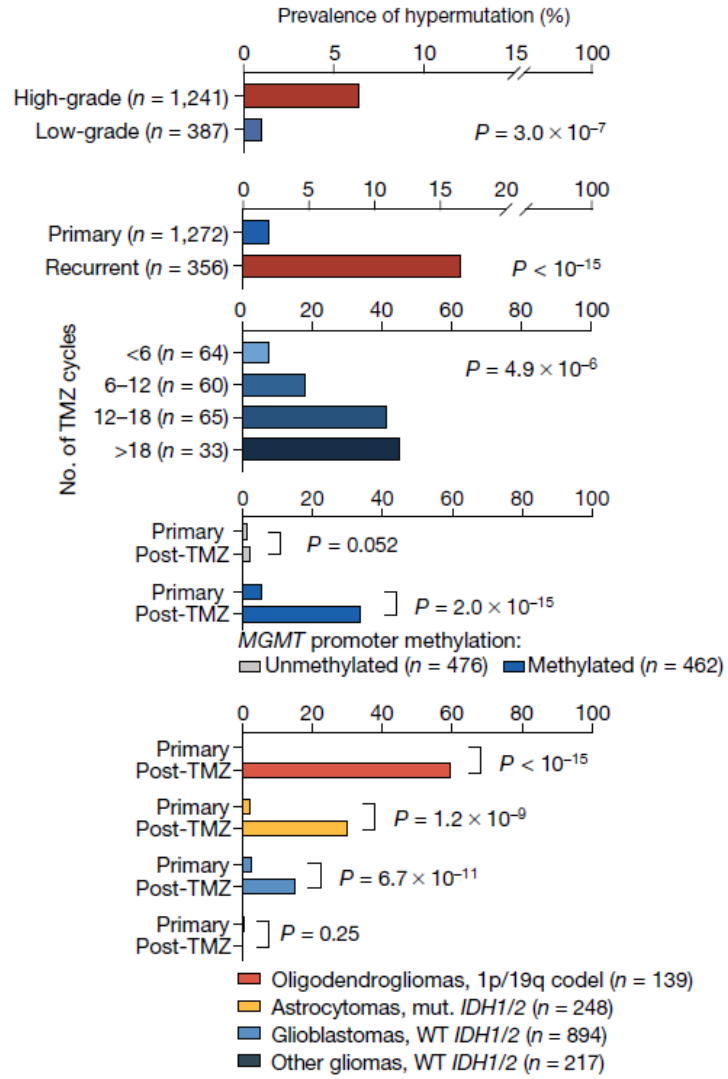
https://doi.org/10.1038/s41586-020-2209-9
Received: 22 July 2019
Accepted: 4 March 2020
Published online: 15 April 2020
Check for updates

Mehdi Touat[1,2,3,34], Yvonne Y. Li[2,4,34], Adam N. Boynton[2,5], Liam F. Spurr[2,4], J. Bryan Iorgulescu[4,6], Craig L. Bohrson[7,8], Isidro Cortes-Ciriano[9], Cristina Birzu[3], Jack E. Geduldig[1], Kristine Pelton[1], Mary Jane Lim-Fat[4,10], Sangita Pal[2,4], Ruben Ferrer-Luna[2], Shakti H. Ramkissoon[11,12], Frank Dubois[2,4], Charlotte Bellamy[1], Naomi Currimjee[4], Juliana Bonardi[1], Kenin Qian[5], Patricia Ho[5], Seth Malinowski[1], Leon Taquet[1], Robert E. Jones[1], Aniket Shetty[13], Kin-Hoe Chow[13], Radwa Sharaf[11], Dean Pavlick[11], Lee A. Albacker[11], Nadia Younan[3], Capucine Baldini[14], Maïté Verreault[15], Marine Giry[15], Erell Guillerm[16], Samy Ammari[17,18], Frédéric Beuvon[19], Karima Mokhtari[20], Agusti Alentorn[3], Caroline Dehais[3], Caroline Houillier[3], Florence Laigle-Donadey[3], Dimitri Psimaras[3], Eudocia Q. Lee[4,10], Lakshmi Nayak[4,10], J. Ricardo McFaline-Figueroa[4,10], Alexandre Carpentier[21], Philippe Cornu[21], Laurent Capelle[21], Bertrand Mathon[21], Jill S. Barnholtz-Sloan[22], Arnab Chakravarti[23], Wenya Linda Bi[24], E. Antonio Chiocca[24], Katie Pricola Fehnel[25], Sanda Alexandrescu[26], Susan N. Chi[5,27], Daphne Haas-Kogan[28], Tracy T. Batchelor[4,10], Garrett M. Frampton[11], Brian M. Alexander[11,28], Raymond Y. Huang[29], Azra H. Ligon[6], Florence Coulet[16], Jean-Yves Delattre[3,30], Khê Hoang-Xuan[3], David M. Meredith[1,6], Sandro Santagata[1,6,31,32], Alex Duval[33], Marc Sanson[3,30], Andrew D. Cherniack[2,4], Patrick Y. Wen[4,10], David A. Reardon[4], Aurélien Marabelle[14], Peter J. Park[7], Ahmed Idbaih[3], Rameen Beroukhim[2,4,10,35], Pratiti Bandopadhayay[2,5,27,35], Franck Bielle[20,35] & Keith L. Ligon[1,2,6,13,26,35]

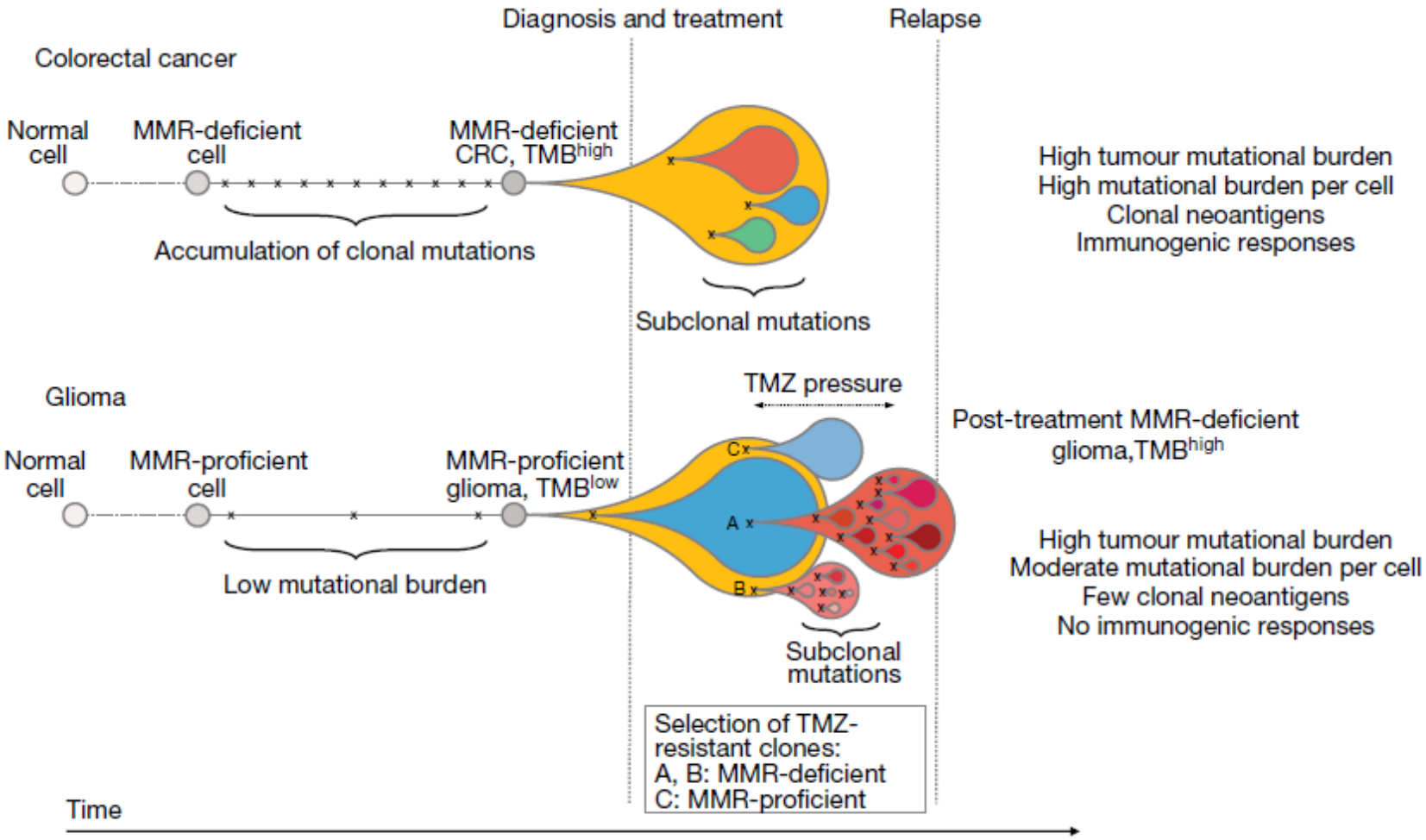
N = 10,294







Model of Differential Response to PD-1 blockade in CRC vs. Glioma



Touat et al. Nature 2020.

Your opinion matters

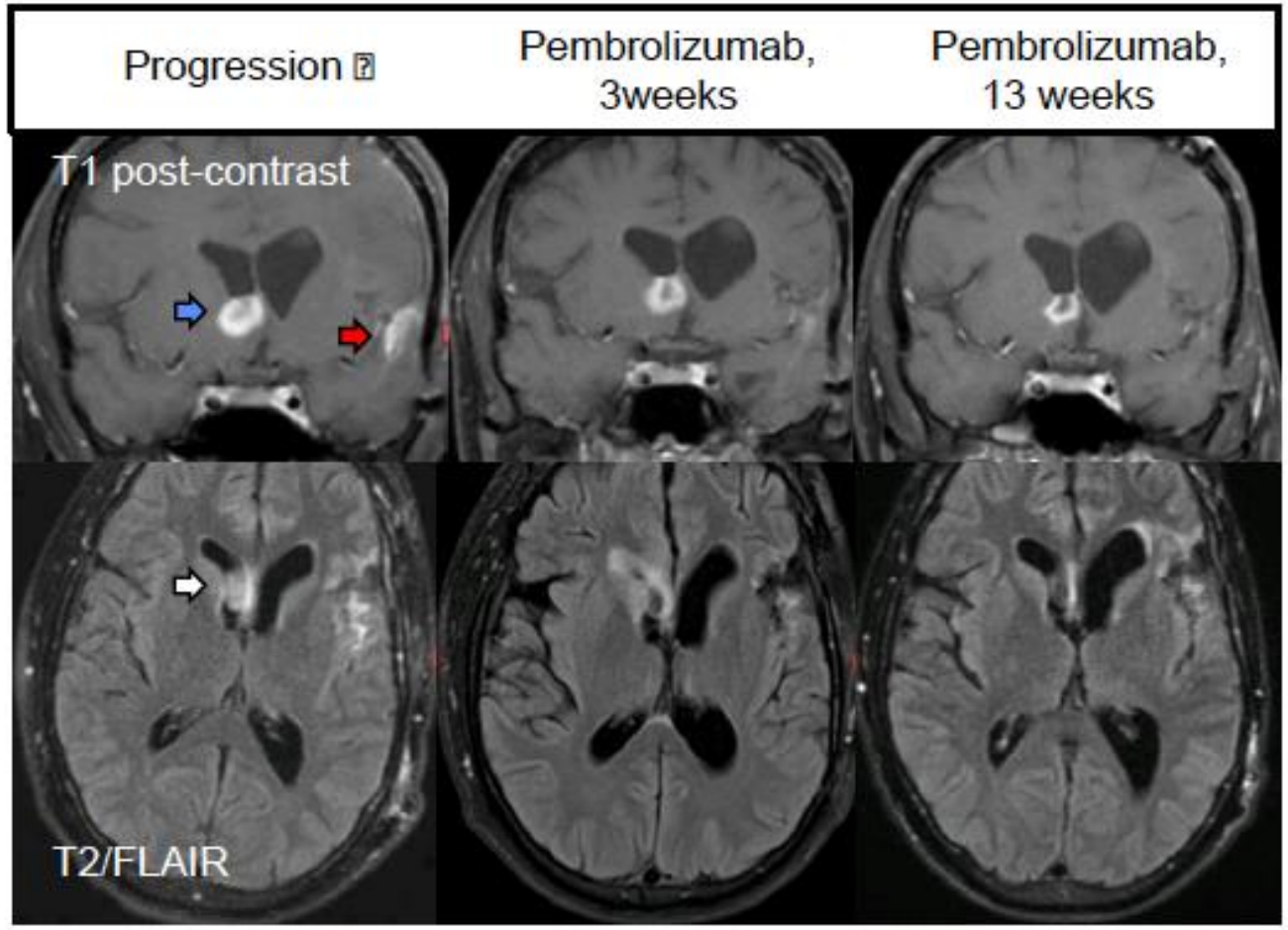
POLL QUESTION #2

FOUNDATION MEDICINE® | Roche

Clinical course

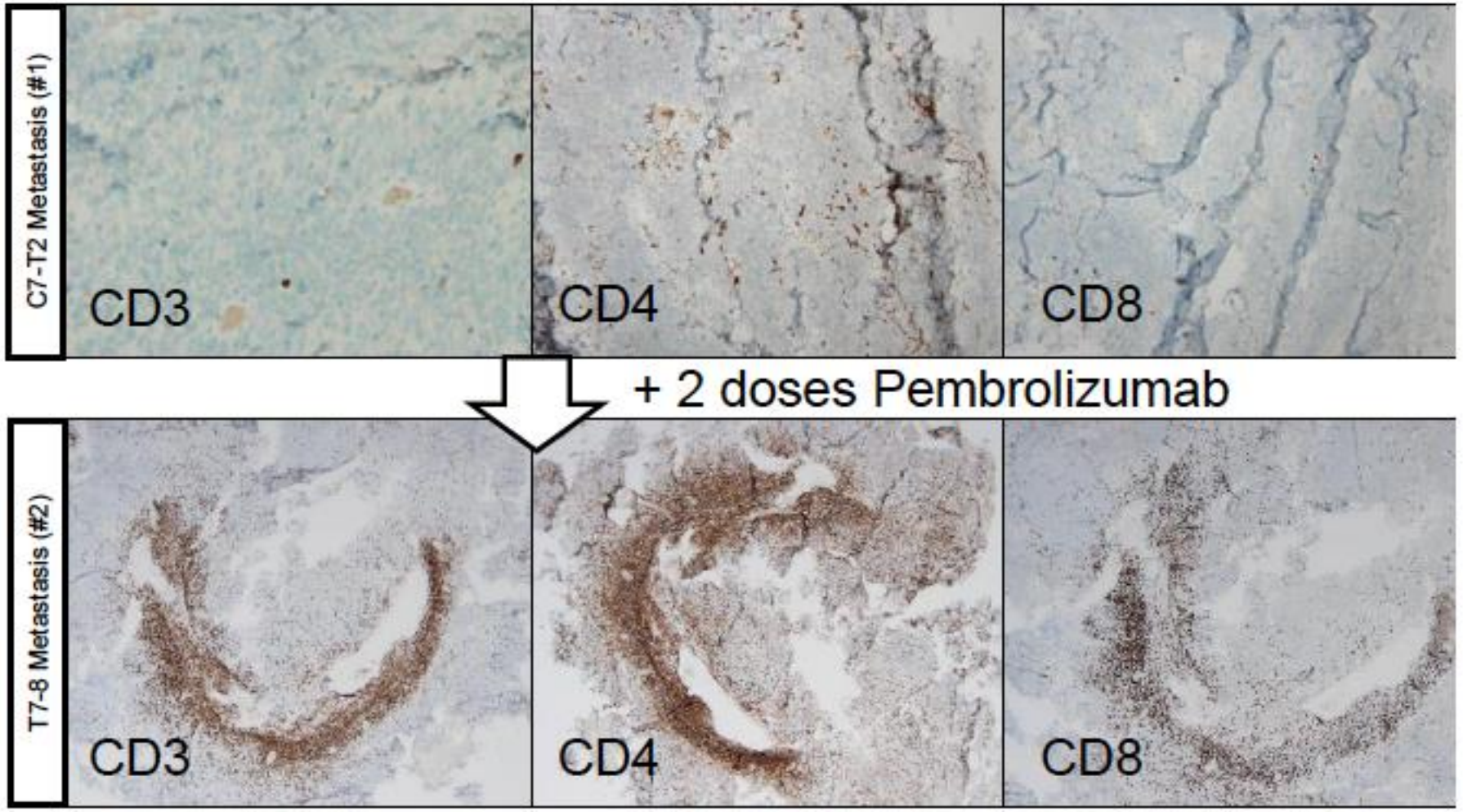
- Recurrent GBM with *POLE* mutation and high tumor mutation burden
- Treatment:
  - Pembrolizumab 200 mg IV every 3 weeks
  - Bevacizumab 500 mg IV every 3 weeks

GBM with Germline POLE Mutation
Response to Pembrolizumab



Johanns et al. Cancer Discov 2016

Cellular Immune Response Following Pembrolizumab Treatment



Johanns et al. Cancer Discov 2016

Journal of Neuro-Oncology (2018) 140:317–328
https://doi.org/10.1007/s11060-018-2955-9

CLINICAL STUDY

Clinical activity and safety of atezolizumab in patients with recurrent glioblastoma

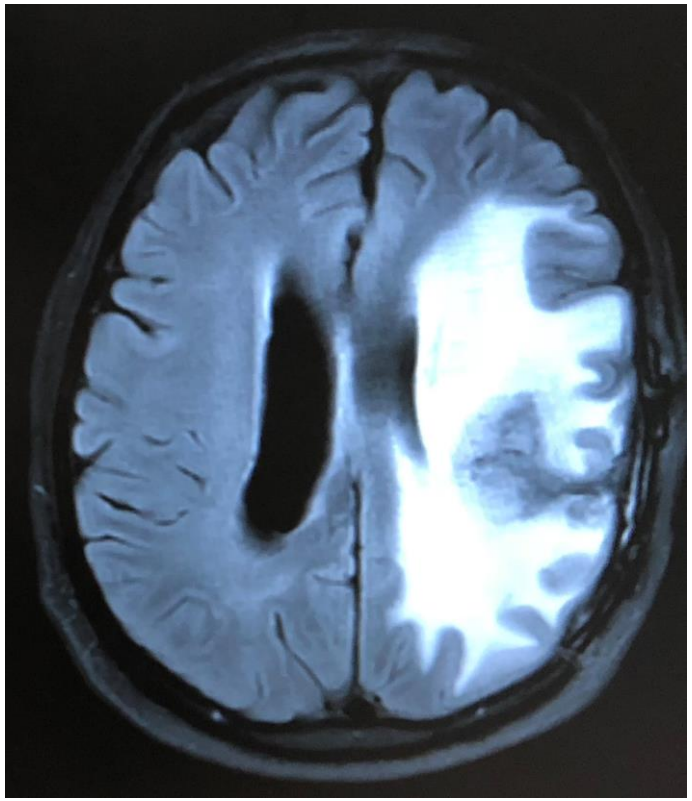
Rimas V. Lukas[1,2] · Jordi Rodon[3] · Kevin Becker[4] · Eric T. Wong[5] · Kent Shih[6] · Mehdi Touat[7] · Marcella Fassò[8] · Stuart Osborne[9] · Luciana Molinero[8] · Carol O'Hear[8] · William Grossman[8] · Joachim Baehring[4]

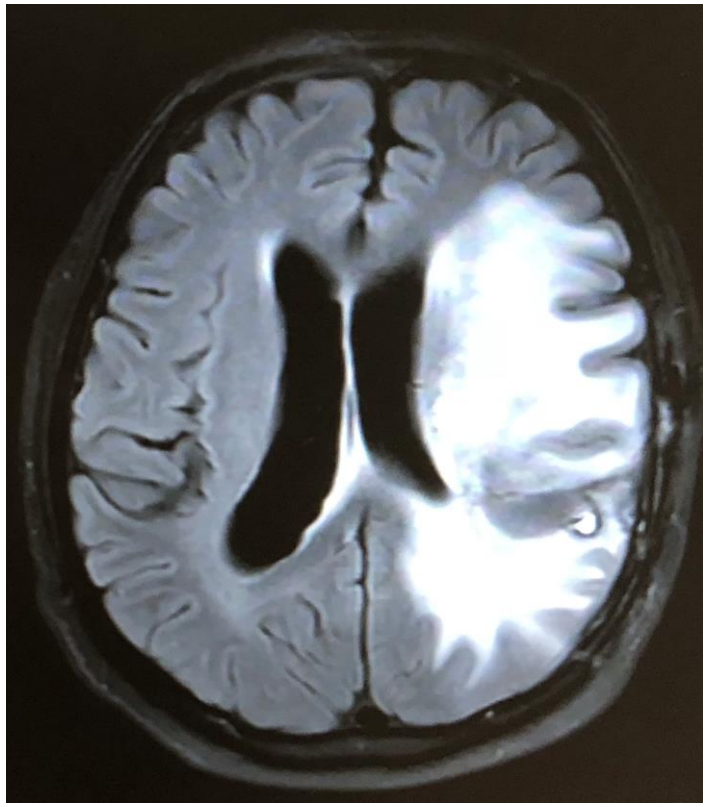
N = 16
Median OS
4.2 months

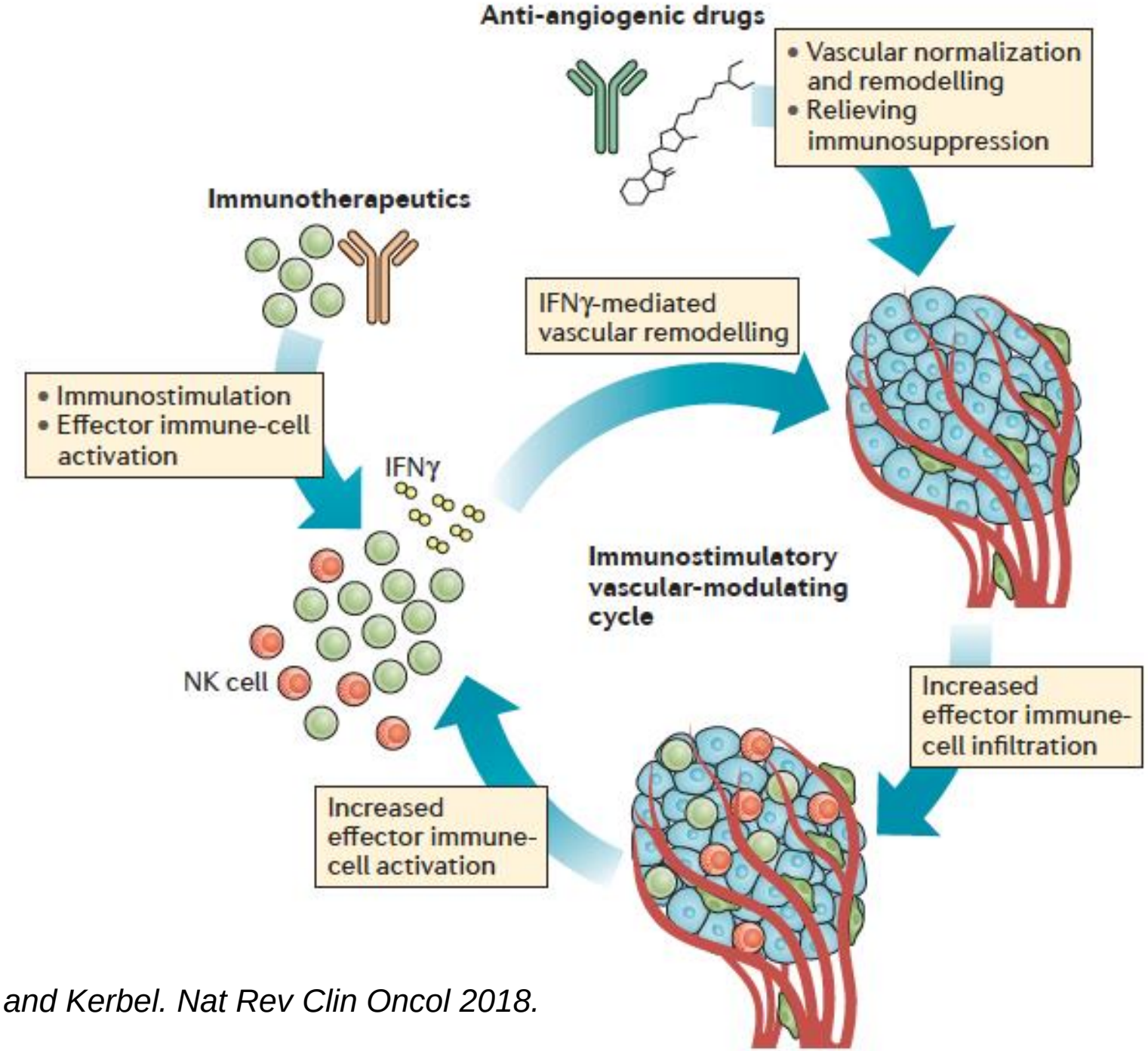
Table 3 Exploratory biomarkers and overall survival

	IDH1 status	*POLE* status	TMB, Mut/Mb	OS, months
Patient 1	Wild-type	L424V	183	17.7+
Patient 2	R132H	Wild-type	0.90	16.0
Patient 3	R132H	Wild-type	2.70	18.8+
Patient 4	R132H	Wild-type	4.50	2.5

Why combining bevacizumab and pembrolizumab in this case?



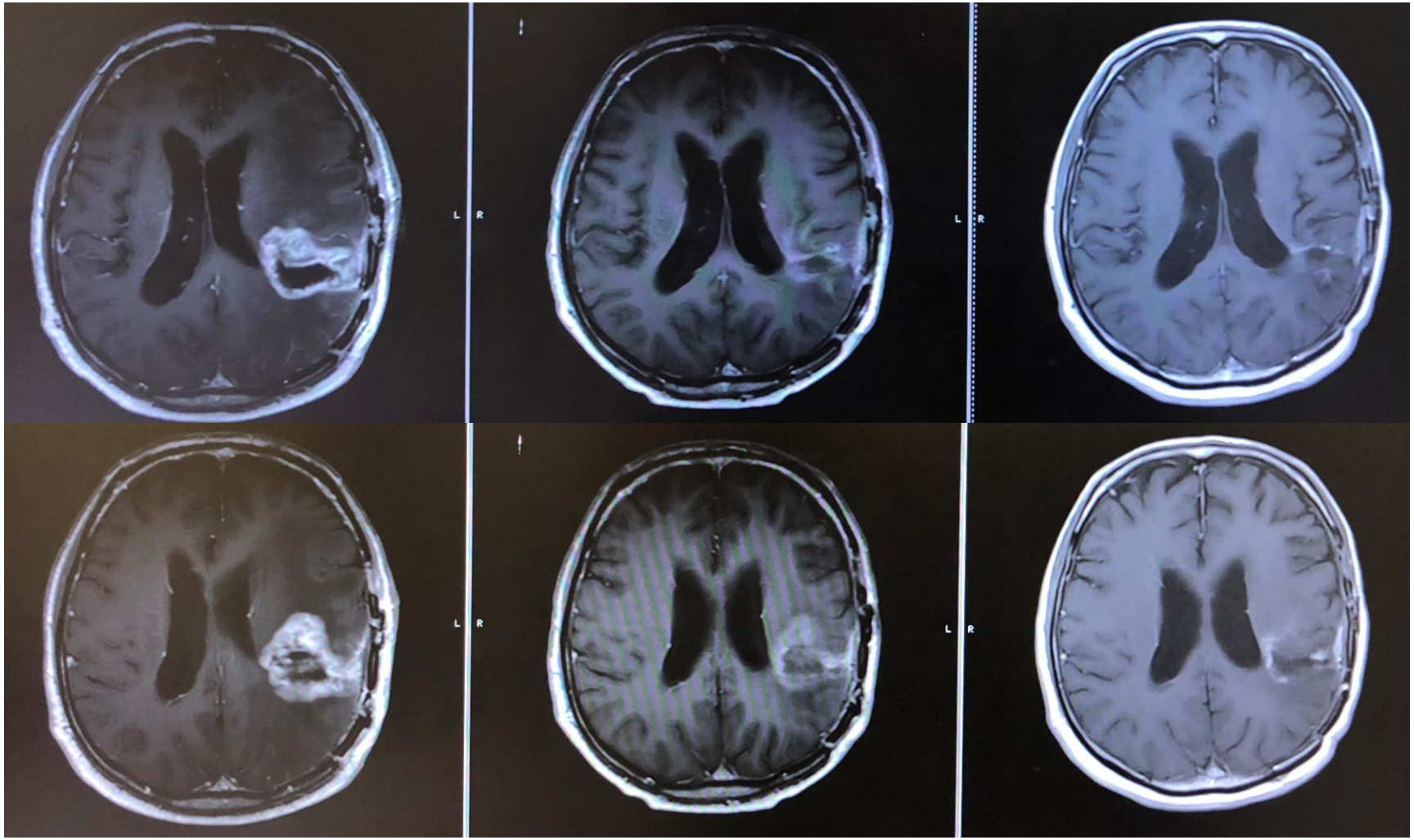




Khan and Kerbel. Nat Rev Clin Oncol 2018.

Recurrent GBM with *POLE* mutation & High TMB

Treatment: Pembrolizumab + Bevacizumab



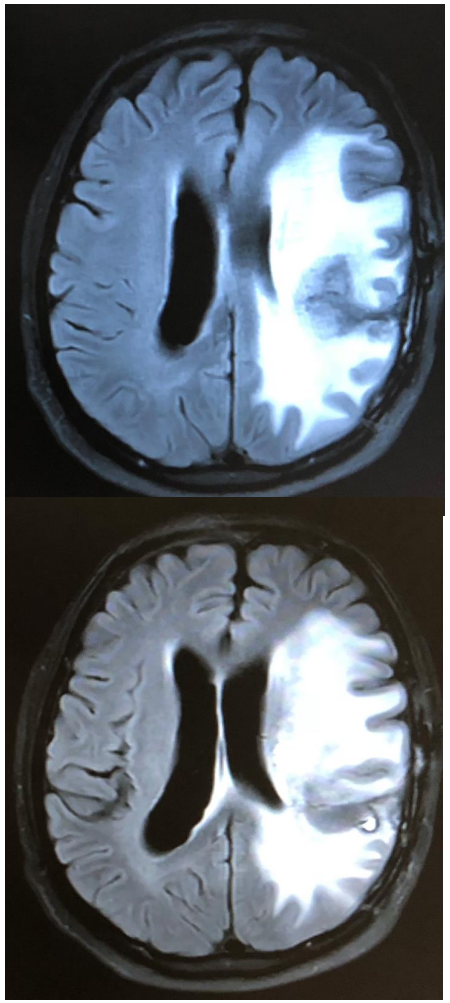
4 weeks post-op.
8/21/17

Pembrolizumab
+ Bevacizumab x 3 months

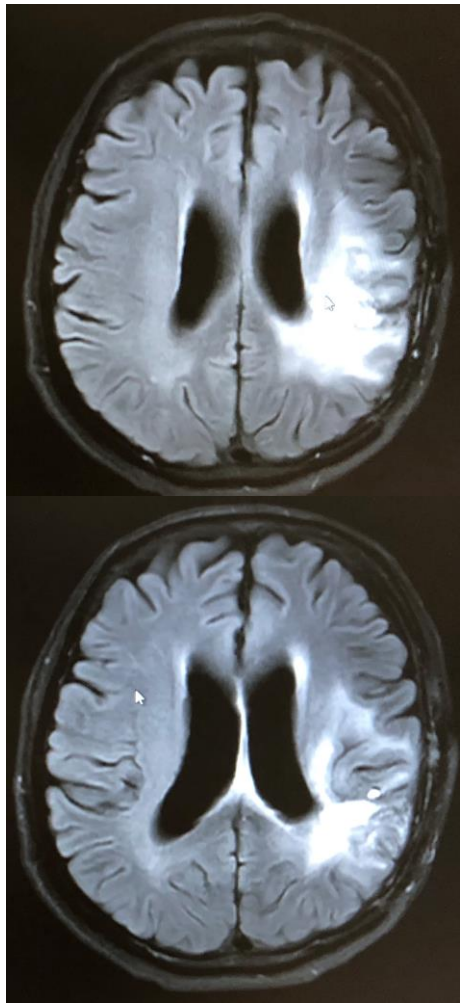
Pembrolizumab
+ Bevacizumab x 12 months

Recurrent GBM with *POLE* mutation & High TMB

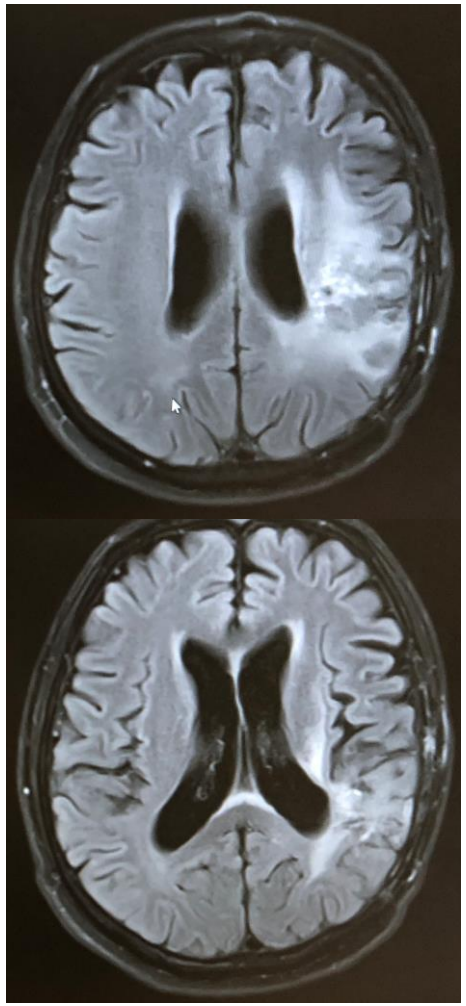
Treatment: Pembrolizumab + Bevacizumab



4 weeks post-op.
8/21/17



Pembrolizumab
+ Bevacizumab x 3 months



Pembrolizumab
+ Bevacizumab x 12 months


Mahidol University
Faculty of Medicine Siriraj Hospital
Thank you