Supplemental Information

Improved overall survival in an anti-PD-L1 treated cohort of newly diagnosed glioblastoma patients is associated with distinct immune, mutation, and gut microbiome features: a single arm prospective phase I/II trial

Shiao-Pei Weathers[1], ^Xiqi Li [2], ^Haifeng Zhu[2], Ashish V. Damania[2,] Mark Knafl[2], Brian McKinley [2,] Heather Lin[3], Rebecca A. Harrison[1], Nazanin K. Majd[1], Barbara J. O'Brien[1], Marta Penas-Prado[1], Monica Loghin[1], Carlos Kamiya-Matsuoka[1], W.K. Alfred Yung[1], Luisa M. Solis Soto[4], Dipen M. Maru[4], Ignacio Wistuba[4], Edwin R. Parra Cuentas[4], Sharia Hernandez[4] Andrew Futreal[2], Jennifer A. Wargo[5], Katja Schulze[6], Walter C. Darbonne [6], Nadim J. Ajami[2], *Scott E. Woodman[2], *John F. de Groot[1]

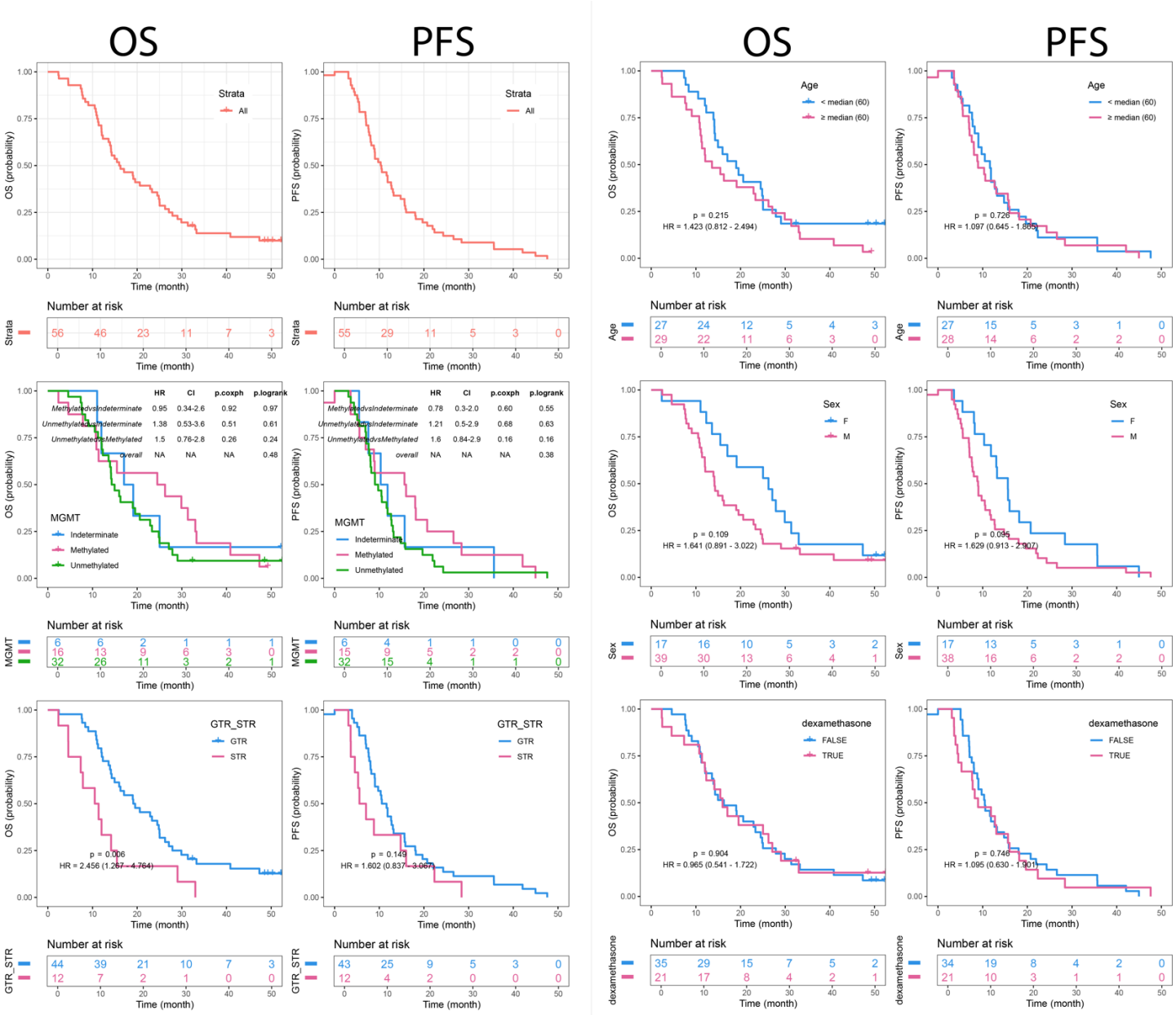
^These authors contributed equally

* These authors jointly supervised this work

[1]Departments of Neuro-Oncology, [2]Genomic Medicine, [3]Biostatistics, [4]Translational Molecular Pathology, [5]Surgical Oncology, The University of Texas MD Anderson Cancer Center, Houston, 1515 Holcombe Blvd, Houston TX 77030. [6]Genentech, Inc 1 DNA Way, San Francisco, CA 94080. Author affiliations at time clinical work was done.

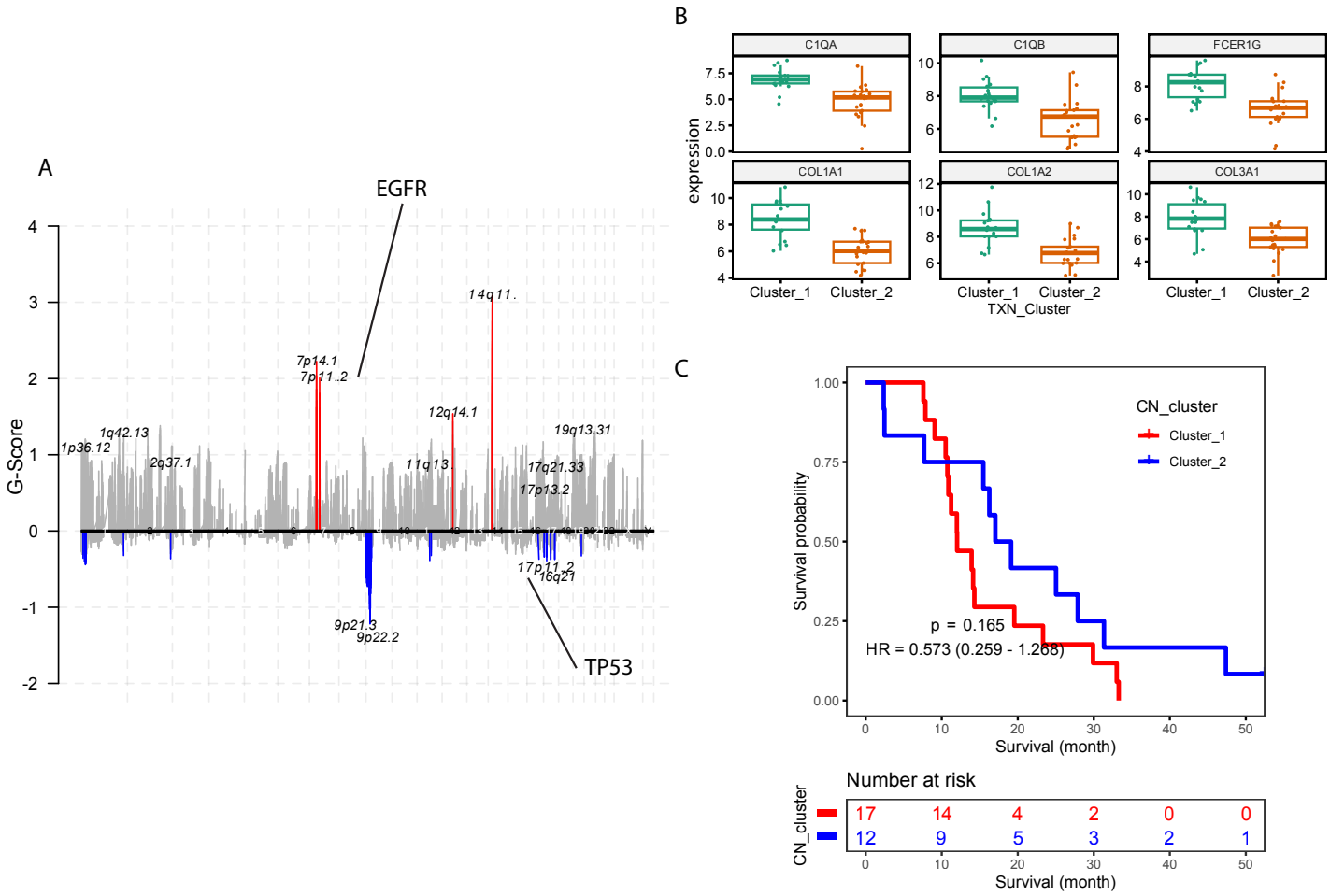
Supplemental Figures

Supplemental Figure 1



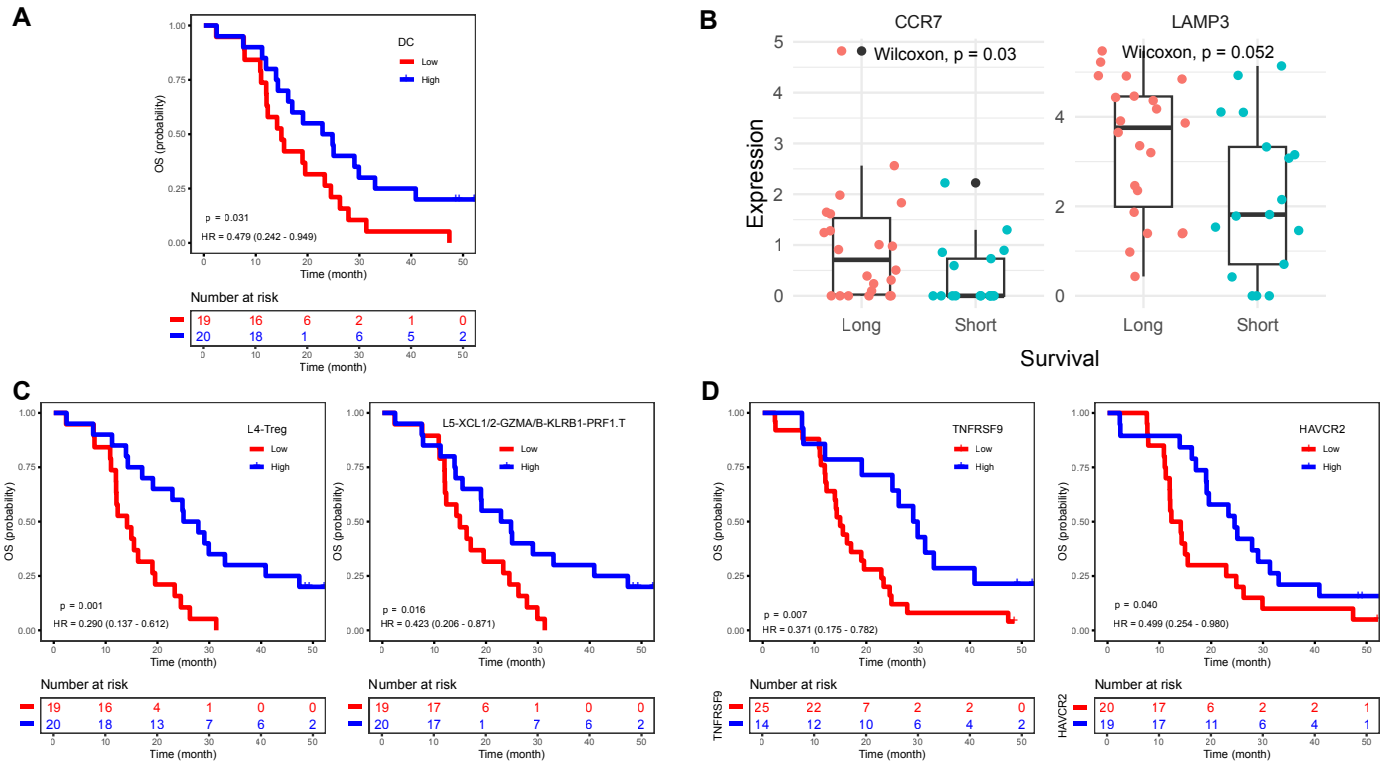
Supplemental Figure 1. PFS and OS Outcomes in Newly Diagnosed GBM Patients Treated with Atezolizimab (anti-PD-L1). Cohort with IDH1 wildtype tumors (n=56). Indeterminant, methylated vs. indeterminant MGMT (n=54). Gross total resection (GTR) vs. subtotal resection (STR) (n=56). Age, low vs. high median (n=56). Sex, male vs. female (n=56). Dexamethasone vs. no dexamethasone (n=56). Unless specified, p-values from the Wald test are displayed.

Supplemental Figure 2



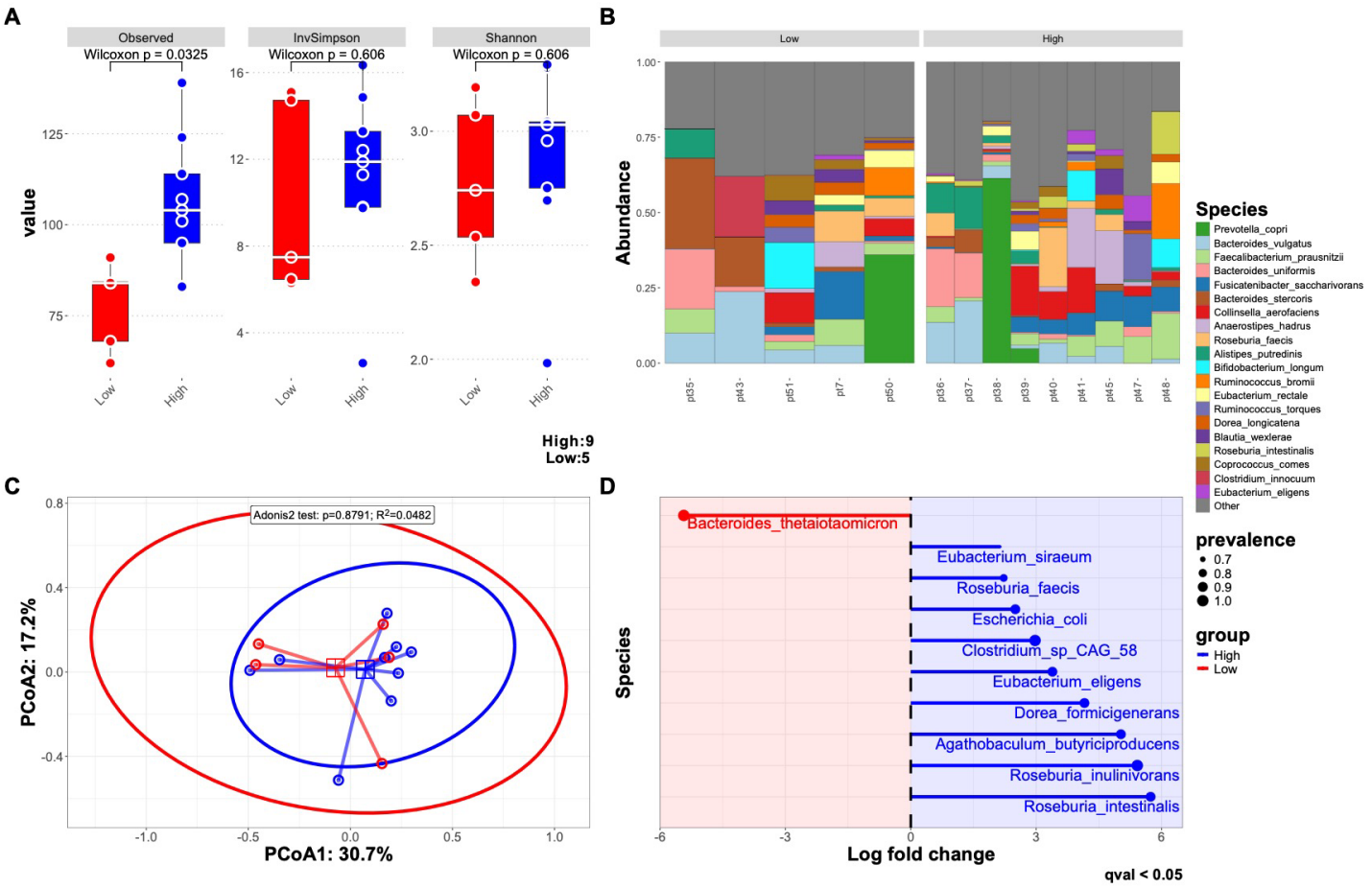
Supplementary Figure 2. Global SCNA Analysis on GBM patients (n=29) and Clinical Outcomes. **A.** Representation of SCNAs in GBM tumors of cohort patients. Source data are provided as a Source Data file. **B.** Box-plot graphs showing differential expression of specific genes between cluster 1 (n=18) and cluster 2 (n=21) samples. **C.** Kaplan-Meier curve of OS for patients based on unsupervised clustering of global SCNA status of GBM tumors. P-value from the Wald test is showed. Source data are provided as a Source Data file.

Supplemental Figure 3



Supplemental Figure 3. Enrichment of scRNAseq-derived myeloid and lymphoid profiles. **A**. Kaplan-Meier curve of OS for patients with low vs. high dendritic cell subcluster profile enrichment score. The p-values from the Wald test are displayed. Source data are provided as a Source Data file. **B**. Box plot representations of "activated and/or migratory" DC subpopulation markers between GBM tumors from patients with short (n=17) vs. long (n=22) OS. **C**. Kaplan-Meier curve of OS for patients with low vs. high L4 (left) and L5 (right) subcluster profile enrichment score. The p-values from the Wald test are displayed. Source data are provided as a Source Data file. **D.** Kaplan-Meier curve of OS for patients with low vs. high immune inhibitory CD4 markers TNFRSF9 (left) and HAVCR2 (right). The p-values from the Wald test are displayed.

Supplemental Figure 4



Supplemental Figure 4. Patient Microbiome is Associated with Tumor Immune Infiltration. **A.** The alpha-diversity of the fecal microbiome was assessed and compared between low (n=5) vs. high (n=9) Estimate Immune Score (EIS) groups. Source data are provided as a Source Data file. **B.** A delineation of the type and relative abundance of each species identified in each patient's fecal microbiome. Source data are provided as a Source Data file. **C.** Principal coordinate analysis of beta-diversity relative to tumor EIS. Source data are provided as a Source Data file. **D.** Prevalence of fecal microbiome bacterial species relative to low vs. high EIS tumor status. Source data are provided as a Source Data file.

Supplemental Tables

Supplemental Table 1

Adverse Event	Grade 3 N (%)	Grade 4 N (%)	Grade 5 N (%)	All Grade N (%)
Lymphocyte count decreased	23 (38.33%)	0 (0%)	0 (0%)	23 (38.33%)
Platelet count decreased	4 (6.67%)	1 (1.67%)	0 (0%)	5 (8.33%)
Alanine aminotransferase increased	2 (3.33%)	2 (3.33%)	0 (0%)	4 (6.67%)
Constipation	3 (5%)	0 (0%)	0 (0%)	3 (5%)
Edema cerebral	0 (0%)	3 (5%)	0 (0%)	3 (5%)
Fatigue	3 (5%)	0 (0%)	0 (0%)	3 (5%)
Aspartate aminotransferase increased	0 (0%)	2 (3.33%)	0 (0%)	2 (3.33%)
Blood bilirubin increased	1 (1.67%)	1 (1.67%)	0 (0%)	2 (3.33%)
Hyperglycemia	2 (3.33%)	0 (0%)	0 (0%)	2 (3.33%)
Neutrophil count decreased	1 (1.67%)	1 (1.67%)	0 (0%)	2 (3.33%)
White blood cell decreased	2 (3.33%)	0 (0%)	0 (0%)	2 (3.33%)
Bone pain	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Confusion	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Lymphocyte count increased	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Muscle weakness lower limb	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Nausea	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Pneumonitis	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)
Urinary tract infection	1 (1.67%)	0 (0%)	0 (0%)	1 (1.67%)

Supplemental Table 1. PFS and OS Outcomes in Newly Diagnosed GBM Patients Treated with Atezolizimab (anti-PD-L1). Cohort with IDH1 wildtype tumors (n=56). Indeterminant, methylated vs. indeterminant MGMT (n=54). Gross total resection (GTR) vs. subtotal resection (STR) (n=56). Age, low vs. high median (n=56). Sex, male vs. female (n=56). Dexamethasone vs. no dexamethasone (n=56).

Supplemental Table 2

Antibody	Catalog #	Vendor	Species
PD1	ab137132	Abcam	Rabbit
CD8	MS-457-S	Thermo Fisher Scientific	Mouse
GFAP	ab68428	Abcam	Rabbit
CD68	M0876	Agilent Dako	Mouse
CD3	A045201-2	Agilent Dako	Rabbit
PD-L1	13684S	Cell Signaling	Rabbit
CD45RO	PA0146	Leica Microsystems	Rabbit
FOXP3	98377S	Cell Signaling	Rabbit
Granzyme B	PA0291	Leica Microsystems	Rabbit

Supplemental Table 2. Sources, catalogue numbers and species of antibodies used in Multiplex Immunoflourescence Imaging.