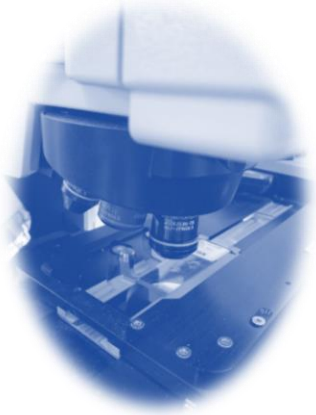
NATIONAL CANCER INSTITUTE
Center for Cancer Research



LABORATORY OF PATHOLOGY

Chief's Letter

06/05/2019

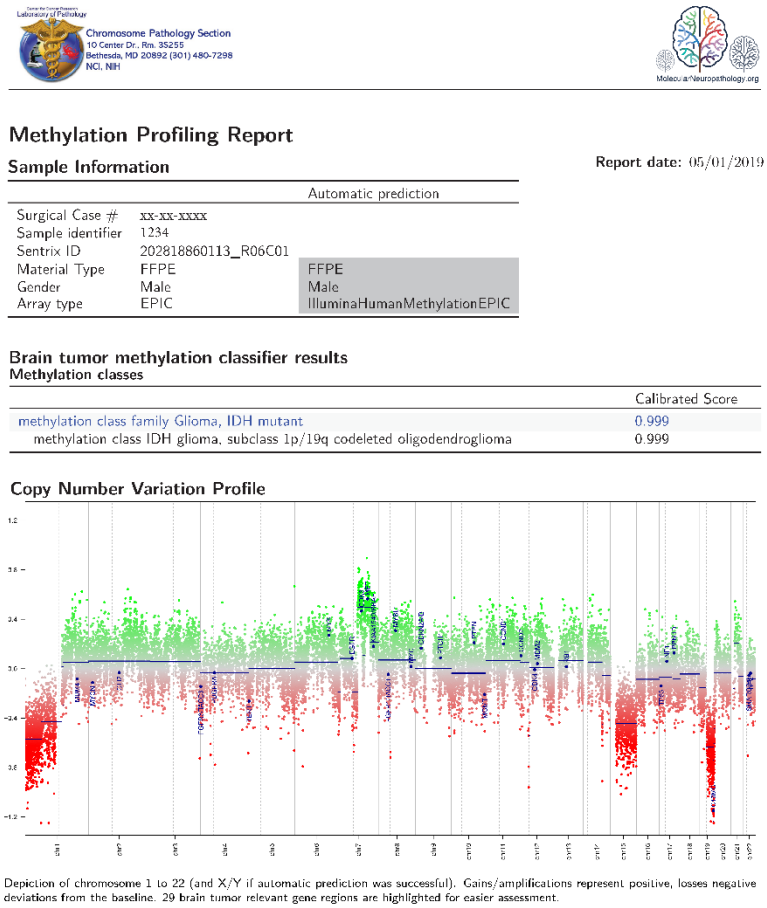
Kenneth Aldape, MD

It is axiomatic that precision medicine in oncology will require advances in precision diagnostics, and advances in our understanding of tumor genomics provide a promising approach towards improvements in precision cancer diagnostics. Individuals bearing tumors with specific molecular alterations, such as gene mutations, amplifications and translocations, and microsatellite instability can be identified using molecular diagnostic tools available in pathology departments, and recent clinical trials for molecularly targeted agents and immunotherapies have been shown to produce unprecedented extended survival in patients with molecularly-defined tumors. While it remains to be determined whether establishing a comprehensive tumor molecular profile of the tumor improves outcome following therapy overall, the evidence to date indicates that this approach represents a promising and dynamic pathway to this goal for specific cancer subsets, and with new data and trials that emerge on an almost a weekly basis, the future remains bright in this area.

In the 20-plus years since I completed my training in anatomic pathology and neuropathology, I believe that the concept of precision cancer diagnostics using tumor molecular profiles represents the most exciting and dynamic opportunity in our field. It is truly an exciting time to be an anatomic pathologist interested in cancer diagnostics. It is with this opportunity and in this context that I joined the Laboratory of Pathology at the National Cancer Institute in Bethesda, Maryland just over one year ago. The past 16 months have been a time of exciting and positive change in our department. Staff dedicated to clinical genomics has more than tripled, and our resource allocation for molecular profiling has dramatically increased. With these resources, we are implementing exciting new technologies in our diagnostic armamentarium. A new 500-gene sequencing panel is set to go live within the next few months and will serve as a standard-of-practice genomic test for solid tumors, to identify actionable mutations, diagnostic fusions and tumor mutational burden/MSI.

In addition to this sequencing platform, we have recently gone live with an exciting new diagnostic tool that uses genome-wide DNA methylation profiling to evaluate tumors of the central nervous system. A recent high-profile publication on this topic, in which I played a collaborative role, showed that tumor methylation profiles can provide definitive evidence to complement and refine morphology-based

diagnostics in tumors of the brain and spinal cord (*example report*➲). We are poised to become a diagnostic reference center to implement this tool for diagnostically challenging neuropathology cases. Going forward, it is likely that new methylation-based classifiers will emerge for additional tumor types and we are poised to lead in this emerging field. Areas of future growth include the implementation of clinical whole-exome sequencing, RNAseq gene expression diagnostics and a liquid biopsy program.



Chromosome Pathology Section
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Molecular Neuropathology.org

Methylation Profiling Report

Sample Information

Report date: 05/01/2019

		Automatic prediction
Surgical Case #	xx-xx-xxxx	
Sample identifier	1234	
Sentrix ID	202818860113_R06C01	
Material Type	FFPE	FFPE
Gender	Male	Male
Array type	EPIC	IlluminaHumanMethylationEPIC

Brain tumor methylation classifier results
Methylation classes

	Calibrated Score
methylation class family Glioma, IDH mutant	0.999
methylation class IDH glioma, subclass 1p/19q codeleted oligodendroglioma	0.999

Copy Number Variation Profile

Depiction of chromosome 1 to 22 (and X/Y if automatic prediction was successful). Gains/amplifications represent positive, losses negative deviations from the baseline. 29 brain tumor relevant gene regions are highlighted for easier assessment.

These advances are not possible without the outstanding work of a dedicated team of scientists and staff who make this possible. An accomplished high-level group, including Liqiang Xi MD, Zied Abdullaev PhD, Francine Blumental De Abreu PhD, Jung Kim PhD, Svetlana Pack PhD and Manoj Tyagi PhD have joined together as a team to leverage their complementary expertise in genomic sequencing, array-based cytogenetics and bioinformatics to lead us into a new era of cancer diagnostics. With this team, we are re-inventing processes to rapidly and accurately determine clinically actionable genomic findings and integrate genomic findings into existing pathology reporting, as well as developing new avenues for research and training of our anatomic pathology residents and fellows.

The field of cancer medicine is moving from an era in which treatment decisions were primarily based on tumor location and histology to a new paradigm whereby treatment decisions in specific clinicopathologic entities will be defined increasingly based on molecular information. We have set the goal to lead the way in the integration of cancer genomics into standard-of-care pathology diagnostics, as well as training the next generation of pathologists to be fully conversant in this area. These are exciting times for anatomic pathologists, and I hope that in this newsletter, as well as those to come, we can describe future advances and accomplishments of our team. With cancers being divided into an ever-increasing number of defined subtypes, a dedicated team-science approach is needed to advance our understanding of these entities and to define ways to effectively treat these cancers. In this spirit, we look forward to hearing from friends, colleagues and alumni of our program to connect and to find collaborative ventures towards a shared goal of improving cancer diagnostics and precision medicine.

The Laboratory of Pathology's Training Programs

Martha Quezado, MD, Director, Anatomic Pathology Residency
Frederic Barr, MD, PhD, Associate Residency Program Director:

The Laboratory of Pathology offers a multifaceted ACGME-accredited residency training program in Anatomic Pathology at the NIH Clinical Center (CC). The CC is the site of intramural clinical research for the NIH, and home to more than 1500 clinical research protocols. Excelling in both clinical diagnosis and translational research, the Laboratory of Pathology provides a stimulating intellectual environment for residents interested in an academic career. The department emphasizes excellence in diagnosis and the use of modern technological tools to enhance accuracy and decipher disease mechanisms. In addition, Laboratory of Pathology staff members receive a large number of cases in consultation each year, resulting in a rich and diversified exposure to the practice of anatomic pathology. Qualified candidates must have completed an M.D. OR D.O. degree from an approved U.S. or Canadian medical school, or must hold an E.C.F.M.G. certificate. Clinical training in the Anatomic Pathology Program includes three years of rotations and subspecialty training. Though several months of research electives are available during the three-year training program, an optional fourth year (or more) may be available to selected residents during which time they will participate in focused research activities with a mentor of their choosing from the many researchers on the NIH campus.

Program Structure

The philosophy of the training program is to provide broad and in-depth exposure to the subject matter of anatomic pathology, with an emphasis on clinical correlation, relationships to disease mechanisms, and exposure to investigational opportunities. Each case under study is viewed in the context of (1) the individual patient's clinical course, (2) strong personal interactions with the clinicians caring for the patient, and (3) the general relevance to disease pathophysiology and investigational questions. Residents become fully grounded in laboratory techniques, observational and descriptive analysis procedures, and the communication skills required to gain the maximum information prior to rendering a diagnosis. The program provides for diversified experience in postmortem pathology, surgical pathology, cytopathology, hematopathology, neuropathology, and molecular pathology. Separate one-month subspecialty rotations in forensic pathology, surgical pathology, and pediatric pathology offered at affiliated institutions broaden the training offered at the NIH. Integrated training in dermatopathology, flow cytometry, immunopathology, informatics, laboratory management, and quality improvement are provided during all three years. Residents in the third year gain more authority in making diagnostic decisions and supervising other residents in both surgical and postmortem pathology.

Stefania Pittaluga MD, PhD, Director, Hematopathology Fellowship

The Hematopathology Section of the Laboratory of Pathology, National Cancer Institute (NCI) offers a fully ACGME accredited fellowship in hematopathology, which provides broad exposure to the

diagnostic and investigative aspects of neoplastic hematopathology. Material is derived principally from an active in-house treatment program for both adult and pediatric hematologic malignancies. In addition, over 2000 challenging cases are submitted in consultation each year. The Hematopathology Section collaborates with the Clinical Services of the NCI, the National Heart, Lung, and Blood Institute, and the National Institute of Allergy and Infectious Diseases in both clinical management and research.

Program Structure

In the first year, fellows focus primarily on clinical rotations, fulfilling the ACGME requirements to qualify for the Board Certification examination in Hematopathology. The majority of time in the second year is available for research, which is an intrinsic component of the fellowship program. Fellows have an opportunity to pursue laboratory interests related to experimental hematopathology and immunology. These investigations include the immunologic, functional, and molecular aspects of human leukemia and lymphoma. Appointments are for a period of two years, with an extension available for additional research time.

Our program is integrated with the Hematology Service of the Department of Laboratory Medicine in the NIH Clinical Center, where fellows receive training in bone marrow pathology and laboratory hematology. Further exposure to laboratory hematology and bone marrow pathology is provided through elective rotations at George Washington University Hospital and Children's National Medical Center. Enhanced exposure to hematopathology and flow cytometry is also obtained through an elective rotation at the Johns Hopkins Hospital.

Armando Filie MD, Director, Cytopathology Fellowship

The Cytopathology Section of the Laboratory of Pathology of the National Cancer Institute provides diagnostic cytopathology services to all institutes of the National Institutes of Health (NIH) and their associated clinical services in the NIH Clinical Center. The relatively high frequency of pathologic findings combined with the diversity of types of exfoliative and fine needle aspiration (FNA) specimens seen in our Section provide a broad experience in diagnostic cytopathology. The Fellowship program is accredited by the Accreditation Council for Graduate Medical Education and is 12 months in duration. For the clinical training, Fellows are assigned to the cytology diagnostic service approximately seventy-five percent (75%) of the time. While on clinical training, the Fellow will have progressive and supervised responsibility for the accessioned cases and for performing superficial FNAs. The Fellow will also rotate for one month each through the Cytopathology Divisions of the Walter Reed National Military Medical Center and the George Washington University Medical Center. Our Section is supported by active and comprehensive immunohistochemistry and molecular pathology services which are utilized for diagnostic and research purposes. The remaining 25% of the time is spent in clinical research training where the Fellow will familiarize him/herself with current techniques that can be applied in the research and clinical setting. The goals of the fellowship are to develop a strong foundation in diagnostic cytopathology and introduce clinically-oriented physicians to current research techniques.

Basic Sciences in the Laboratory of Pathology

The basic science of pathology rests on the foundational pillars of biology, physiology, biochemistry, molecular biology, genetics, immunology, chemistry and physics. The Basic Science PIs and staff of the Laboratory of Pathology are drawing upon their knowledge of these subjects and their diverse expertise and talents to make cross-disciplinary contributions that illuminate the pathogenesis of cancer, that define the body's response to neoplasia and that highlight new strategies to intercept and reverse disease progression.

The lab of **Dave Roberts, PhD**, has been a leader in defining the complex cross-talk between tumors and the immune system. His team has interrogated the role and connections of the cell-surface protein CD47 as an immune modulator that controls both tumor cells and responding lymphocytes and macrophages. Though the bulk of the literature has focused on ligand-receptor actions of tumor cell CD47 delivering a "don't eat me signal" through TSP-1 to the immune system, the Roberts group has painted a more intricate picture of actions of these molecules. Recently, they have seen a convergence of approaches that study the protein partners of CD47 and approaches that have examined the RNA cargoes of extracellular vesicles (exosomes) derived from cells with or without CD47. Unexpectedly, CD47 associated with components of the nuclear export complex that shepherds RNAs from the nucleus to the cytoplasm. The RNAs shepherded by CD47 are the same species that depend on CD47 for localization to exosomes; thus, CD47 seems to be a versatile tool for governing cell fate.

Speaking of exosomes, we have recently been joined by **Jennifer Jones, MD, PhD**, who is developing new strategies to enrich and characterize extracellular vesicles. This work promises to enable new strategies for the diagnosis and submicroscopic analysis of small and remote tumors and has the potential to instruct strategies that use engineered exosomes to deliver therapeutic payloads to tumors.

Fred Barr, MD PhD, has continued his exploration of the molecular mechanisms that drive the pathogenesis and clinical evolution of the pediatric soft tissue cancer rhabdomyosarcoma. Beyond the chromosomal rearrangements that he discovered, he is now exploring the interplay between driver mutations and epigenetics as he investigates how DNA methylation influences behavior in this family of soft tissue cancers.

Eric Batchelor, PhD, and David Levens, MD, PhD have continued to explore the roles and mechanisms of the TP53 tumor suppressor and MYC oncoprotein, respectively, as well as to identify how the interplay between these two systems alters cell behavior in response to DNA damage. The interaction between these molecules and these investigators, along with Jay Schneekloth, PhD, CBL, catalyzed a CCR symposium, "Frontiers in Targeting MYC: Expression, Regulation and Degradation". This meeting brought together diverse scientists to present their results and to brainstorm about how to therapeutically target what may be the most frequently deregulated oncogene in human cancer.

The Batchelor lab continues to elucidate the mechanisms and consequences of dynamic changes, especially oscillations, in the expression of TP53. Such changes may directly influence the therapeutic efficacy of radiation and chemotherapy.

Dave Levens' group continues to explore the interplay between DNA topology, DNA structure and gene regulation. With Brian Lewis and Fedor Kouzine and the other members of the Gene Regulation Section, the goal of elucidating dynamic and concerted changes in the organization of the transcription machinery and in DNA topology under high output conditions such as occurs with high levels of MYC.

We are pleased to announce, after an extended sojourn in the Radiation Oncology Branch, **Bill Stetler-Stevenson, MD, PhD**, an expert in the biology and biochemistry of metalloproteinases and their inhibitors, which regulate and remodel the extracellular matrix through which tumor cells invade and migrate, has returned to the Laboratory of Pathology.

Besides leading an initiative to classify brain tumors according to DNA methylation patterns, the Aldape laborator is studying CIC, a fascinating and understudied molecular miscreant on chromosome 19 that is frequently deleted in low-grade gliomas (along with FUBP1 on chromosome 1 - which has been extensively studied in the Levens lab). The goal of the Aldape lab will be to build upon our recent publication demonstrating that CIC contributes to tumorigenesis in glioblastoma, as well as to continue to explore clinically relevant genomic alterations in gliomas and meningiomas.

Awards

We are proud to share news of the 2019 USCAP Board's Distinguished Pathologist Award to Dr. Elaine S. Jaffe.

Dr. Jaffe studied at Cornell University Medical College and the University of Pennsylvania, receiving her M.D. degree from the latter. Her residency and fellowship in pathology were completed at the National Cancer Institute, where she went on to become Head of the Hematopathology Section of the Laboratory of Pathology. One of her earliest papers on follicular lymphoma (1974), a Citation Classic, presented evidence for the origin of this tumor from follicular B cells. More recently she described in situ follicular lymphomas, which provide insight into the earliest events of follicular lymphomagenesis and identified histiocytic/dendritic cell tumors arising in follicular lymphoma, providing the first evidence for lineage plasticity in mature human lymphoid cells. She has authored more than 600 peer reviewed articles, 40 invited editorials, and more than 150 chapters and invited reviews.

Dr. Jaffe was an Editor for the 2001 and 2008 editions of the WHO Classification of Tumors of the Hematopoietic and Lymphoid Tissues and was one of four series editors overseeing the 4th Edition Series of Bluebooks for the classification of human tumors, under the auspices of the International Agency for Research on Cancer and the World Health Organization. Her textbook, Hematopathology, is now in its second edition. Dr. Jaffe has served on 21 journal editorial boards, including the USCAP journals, Modern Pathology and Laboratory Investigation. Science Watch named her among the 10 most highly cited researchers in clinical oncology from 1981 and 1998, and she was among the 400 most highly cited researchers in all Biomedical Sciences worldwide between 1996 and 2011. She is past

president of both the Society for Hematopathology and USCAP. She has held several leadership positions in the American Society of Hematology.

Among her awards are the Fred W. Stewart Award from Memorial-Sloan Kettering Cancer Center, the Lennert prize from the European Association for Haematopathology, the Maude Abbott Lecturer for the United States and Canadian Academy of Pathology, the Stratton Medal from the American Society of Hematology, and the Rous Whipple Award from the American Society of Investigative Pathology. Dr. Jaffe was recognized for her mentoring with awards from the National Cancer Institute and the American Society of Investigative Pathology. She received an honorary degree from the University of Barcelona, and the Distinguished Graduate Award from the University of Pennsylvania School of Medicine in 2011. In 1993, Dr. Jaffe was elected a fellow of the American Association for the Advancement of Science, and subsequently was elected Chair of the Medical Sciences Section of AAAS. She was elected to the Institute of Medicine of the National Academies – now the National Academy of Medicine - and currently serves as Membership Chair for Section 4 (Internal Medicine, Pathology and Dermatology).

Selected Recent Publications by Laboratory of Pathology Faculty

1. Relationship of DNA methylation to mutational changes and transcriptional organization in fusion-positive and fusion-negative rhabdomyosarcoma. Sun W, Chatterjee B, Shern JF, Patidar R, Song Y, Wang Y, Walker RL, Pawel BR, Linardic CM, Houghton P, Hewitt SM, Edelman DC, Khan J, Meltzer PS, **Barr FG**. Int J Cancer. 2019 Jun 1;144(11):2707-2717. doi: 10.1002/ijc.32006. Epub 2019 Jan 15. PMID: 30565669.
2. Mapping DNA Breaks by Next-Generation Sequencing. Baranello L, Kouzine F, Wojtowicz D, Cui K, Zhao K, Przytycka TM, Capranico G, **Levens D**. Methods Mol Biol. 2018;1672:155-166. doi: 10.1007/978-1-4939-7306-4_13. PMID: 29043624
3. CIC protein instability contributes to tumorigenesis in glioblastoma. Bunda S, Heir P, Metcalf J, Li ASC, Agnihotri S, Pusch S, Yasin M, Li M, Burrell K, Mansouri S, Singh O, Wilson M, Alamsahebpour A, Nejad R, Choi B, Kim D, von Deimling A, Zadeh G, **Aldape K**. Nature Communications. 2019 Feb 8;10(1):661. doi: 10.1038/s41467-018-08087-9. PMID: 30737375.
4. Expression of ALCAM (CD166) and PD-L1 (CD274) independently predicts shorter survival in malignant pleural mesothelioma. Inaguma S, Lasota J, Wang Z, Czapiewski P, Langfort R, Rys J, Szpor J, Waloszczyk P, Okoń K, Biernat W, Ikeda H, Schrump DS, Hassan R, **Miettinen M**. Hum Pathol. 2018 Jan;71:1-7. doi: 10.1016/j.humpath.2017.04.032. Epub 2017 Aug 12. PMID: 28811252.
5. Expression of CD70 (CD27L) Is Associated with Epithelioid and Sarcomatous Features in IDH-Wild-Type Glioblastoma. Pratt D, Pittaluga S, Palisoc M, Fetsch P, Xi L, Raffeld M, Gilbert MR, **Quezado M**. J Neuropathol Exp Neurol. 2017 Aug 1;76(8):697-708. doi: 10.1093/jnen/nlx051. PMID: 28789475.
6. Hepatocellular carcinoma: Liver biopsy in the balance. **Kleiner DE**. Hepatology. 2018 Jul;68(1):13-15. doi: 10.1002/hep.29831. PMID: 29405373.

7. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, **Jaffe ES**. Blood. 2019 Apr 18;133(16):1703-1714. doi: 10.1182/blood-2018-11-881268. Epub 2019 Jan 11. Review. PMID: 30635287.
8. A Study of PD-L1 Expression in Intravascular Large B-cell Lymphoma: Correlation with Clinical and Pathologic Features. Gupta GK, Jaffe ES, **Pittaluga S**. Histopathology. 2019 Apr 2. doi: 10.1111/his.13870. [Epub ahead of print] PMID: 30938862.
9. Protein stability of p53 targets determines their temporal expression dynamics in response to p53 pulsing. Hanson RL, Porter JR, **Batchelor E**. J Cell Biol. 2019 Apr 1;218(4):1282-1297. doi: 10.1083/jcb.201803063. Epub 2019 Feb 11. PMID: 30745421.
10. Correlation between ERG Fusion Protein and Androgen Receptor Expression by Immunohistochemistry in Prostate, Possible Role in Diagnosis and Therapy. Navaei AH, Walter BA, Moreno V, Pack SD, Pinto P, **Merino MJ**. J Cancer. 2017 Aug 5;8(13):2604-2613. doi: 10.7150/jca.16751. eCollection 2017. PMID: 28900498.
11. Quantification of B-cell maturation antigen, a target for novel chimeric antigen receptor T-cell therapy in Myeloma. Salem DA, Maric I, Yuan CM, Liewehr DJ, Venzon DJ, Kochenderfer J, **Stetler-Stevenson M**. Leuk Res. 2018 Aug;71:106-111. doi: 10.1016/j.leukres.2018.07.015. Epub 2018 Jul 18. PMID: 30053652.
12. Early recovery of circulating immature B cells in B-lymphoblastic leukemia patients after CD19 targeted CAR T cell therapy: A pitfall for minimal residual disease detection. Xiao W, Salem D, McCoy CS, Lee D, Shah NN, Stetler-Stevenson M, **Yuan CM**. Cytometry B Clin Cytom. 2018 May;94(3):434-443. doi: 10.1002/cyto.b.21591. Epub 2017 Oct 31. PMID: 28888074.
13. CD63, MHC class 1, and CD47 identify subsets of extracellular vesicles containing distinct populations of noncoding RNAs. Kaur S, Elkahloun AG, Arakelyan A, Young L, Myers TG, Otaizo-Carrasquero F, Wu W, Margolis L, **Roberts DD**. Sci Rep. 2018 Feb 7;8(1):2577. doi: 10.1038/s41598-018-20936-7. PMID: 29416092.
14. Elevated expression of pancreatic adenocarcinoma upregulated factor (PAUF) is associated with poor prognosis and chemoresistance in epithelial ovarian cancer. Choi CH, Kang TH, Song JS, Kim YS, Chung EJ, Ylaya K, Kim S, Koh SS, Chung JY, Kim JH, **Hewitt SM**. Sci Rep. 2018 Aug 15;8(1):12161. doi: 10.1038/s41598-018-30582-8. PMID: 30111860.
15. Molecular assessment of clonality in lymphoid neoplasms. **Wang HW, Raffeld M**. Semin Hematol. 2019 Jan;56(1):37-45. doi: 10.1053/j.seminhematol.2018.05.008. Epub 2018 May 26. Review. PMID: 30573043.
16. Labeling Extracellular Vesicles for Nanoscale Flow Cytometry. Morales-Kastresana A, Telford B, Musich TA, McKinnon K, Clayborne C, Braig Z, Rosner A, Demberg T, Watson DC, Karpova TS, Freeman GJ, DeKruyff RH, Pavlakis GN, Terabe M, Robert-Guroff M, Berzofsky JA, **Jones JC**. Sci Rep. 2017 May 12;7(1):1878. doi: 10.1038/s41598-017-01731-2. PMID: 28500324.
17. Macromolecule-Network Electrostatics Controlling Delivery of the Biotherapeutic Cell Modulator TIMP-2. Yamada Y, Chowdhury A, Schneider JP, **Stetler-Stevenson WG**. Biomacromolecules. 2018 Apr 9;19(4):1285-1293. doi: 10.1021/acs.biomac.8b00107. Epub 2018 Mar 19. PMID: 29505725