HIGH IMPACT PUBLICATIONS WITH THE HALO® IMAGE ANALYSIS PLATFORM

Digital pathology with the HALO image analysis platform continues to advance research areas as diverse as immunology, infectious disease, immuno-oncology, and neuroscience. Here we highlight recent high-impact publications according to research area. Although many publications could have been placed into several categories, they are only listed once. Check any related categories for additional publications in your research area.

IMMUNOLOGY & INFECTIOUS DISEASE

Carvelli J, Demaria O, Vély F et *al.* Association of COVID-19 inflammation with activation of the C5a–C5aR1 axis. *Nature* **588**: 146–150 (2020). DOI: 10.1038/s41586-020-2600-6.

Giannakis N, Sansbury BE, Patsalos A et *al.* Dynamic changes to lipid mediators support transitions among macrophage subtypes during muscle regeneration. *Nat Immunol* **20**: 626–636 (2019). DOI: 10.1038/s41590-019-0356-7.

Harper J, Gordon S, Chan CN et *al.* CTLA-4 and PD-1 dual blockade induces SIV reactivation without control of rebound after antiretroviral therapy interruption. *Nat Med* **26**: 519–528 (2020). DOI: 10.1038/s41591-020-0782-y

McNairn AJ, Chuang CH, Bloom JC et *al.* Female-biased embryonic death from inflammation induced by genomic instability. *Nature* **567**: 105–108 (2019). DOI: 10.1038/s41586-019-0936-6.

Wang Y, Lifshitz L, Gellatly K. et *al.* HIV-1-induced cytokines deplete homeostatic innate lymphoid cellsand expand *TCF7*-dependent memory NK cells. *Nat Immunol* **21**: 274–286 (2020). DOI: 10.1038/s41590-020-0593-9.

CXCR3 ENABLES RECRUITMENT AND SITE-SPECIFIC BYSTANDER ACTIVATION OF MEMORY CD8+ T CELLS

Authors: NJ Maurice, MJ McElrath, E Andersen-Nissen, N Frahm, M Prlic

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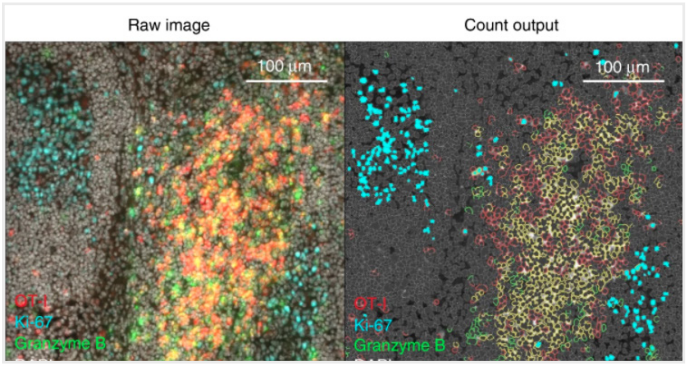

Science: Bystander activation is the phenomenon by which memory T cells can be activated in an inflammation dependent but antigen independent fashion. Nicholas Maurice and colleagues demonstrate a molecular mechanism of bystander activation, and their research suggests that it occurs during initial localized immune responses and is not limited to induction by systemic inflammation.

Technology: HALO was used to enumerate the CD8+ T cells (OT-I cells) in whole-spleen sections as well as Ki-67+ and granzyme B+ OT-I cells. In Figure 2c shown above, immunofluorescence (IF) images are shown with staining specific for OT-I cells (red), Ki-67 (cyan), Granzyme B (green), and DAPI (white).

IMMUNO-ONCOLOGY

Bakouny Z, Braun DA, Shukla SA et *al.* Integrative molecular characterization of sarcomatoid and rhabdoid renal cell carcinoma. *Nat Commun* **12**: 808 (2021). DOI: 10.1038/s41467-021-21068-9.

Gao J, Navai N, Alhalabi O. et *al.* Neoadjuvant PD-L1 plus CTLA-4 blockade in patients with cisplatin-ineligible operable high-risk urothelial carcinoma. *Nat Med* **26**: 1845–1851 (2020). DOI: 10.1038/s41591-020-1086-y.

Goto M, Shibahara Y, Baciu C et *al.* Prognostic Impact of CXCR7 and CXCL12 Expression in Patients with Esophageal Adenocarcinoma. *Ann Surg Oncol* **Mar 11** (2021). DOI: 10.1245/s10434-021-09775-5.

Guo C, Crespo M, Gurel B et *al.* CD38 in Advanced Prostate Cancers. *Eur Urol.* **79**: 736-746 (2021). DOI: 10.1016/j.eururo.2021.01.017.

Jackstadt R, van Hooff SR, Leach, JD et *al.* Epithelial NOTCH Signaling Rewires the Tumor Microenvironment of Colorectal Cancer to Drive Poor-Prognosis Subtypes and Metastasis. *Cancer Cell* **36**: 319-336.e7 (2019). DOI: 10.1016/j.ccell.2019.08.003.

Reyes-Uribe L, Wu W, Gelincik O, et *al.* Naproxen chemoprevention promotes immune activation in Lynch syndrome colorectal mucosa. *Gut* **70**: 555-566 (2020). DOI: 10.1136/gutjnl-2020-320946.

PERSISTENCE OF ADOPTIVELY TRANSFERRED T CELLS WITH A KINETICALLY ENGINEERED IL-2 RECEPTOR AGONIST

Authors: G Parisi, JD Saco, FB Salazar, J Tsoi, P Krystofinski, C Puig-Saus, R Zhang, J Zhou, GC Cheung-Lau, AJ Garcia, CS Grasso, R Tavaré, S Hu-Lieskovan, S Mackay, J Zalevsky, C Bernatchez, A Diab, AM Wu, B Comin-Anduix, D Charych, A Ribas

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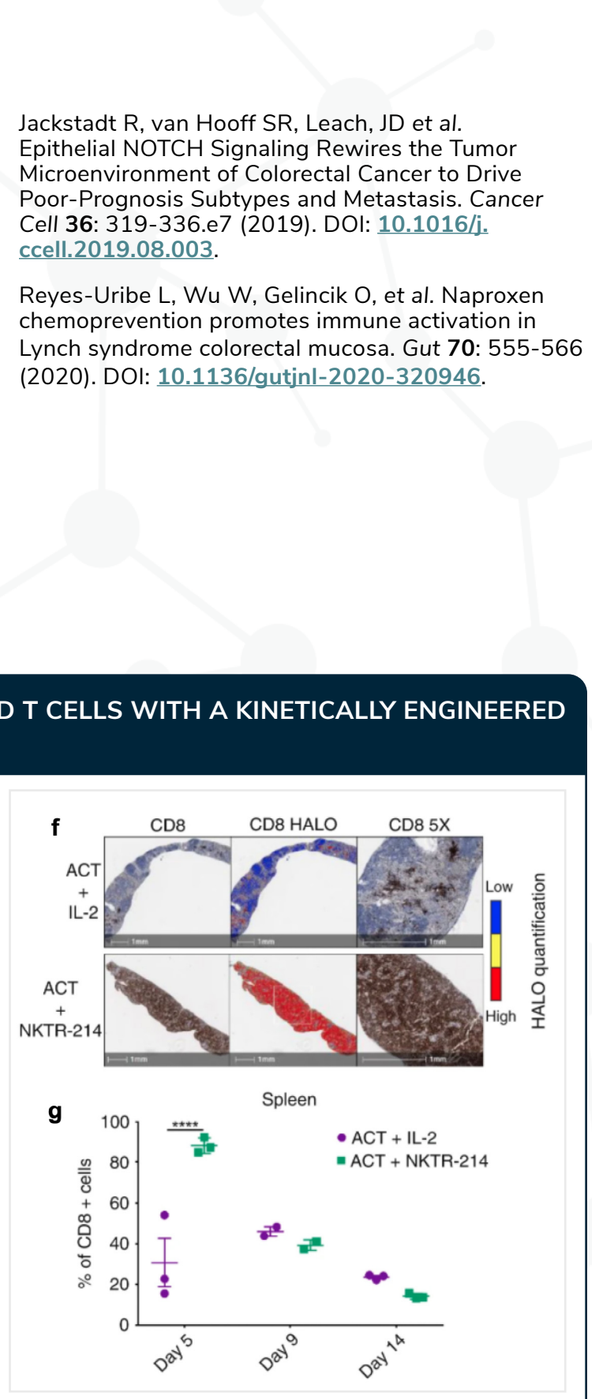
Science: T-cell therapies are commonly given with high doses of interleukin 2 (IL-2) to support the transferred T cells, however, this dose of IL-2 is associated with acute toxicity. Giulia Parisi and team demonstrate the use of a kinetically engineered IL-2 receptor (IL-2Rβγ)-biased agonist that outperforms the antitumor activity of IL-2 in a preclinical model. Other studies preliminarily demonstrate a favorable safety profile, lending hope that a clinical replacement of high doses of IL-2 are on the horizon for patients receiving T-cell therapies.



Technology: HALO was used to quantify CD8 positivity in immunohistochemistry analysis of spleen, tumor, kidney, and liver tissue samples. The CD8 HALO portion of Figure 2F shown above is a heat map of CD8+ T cells. Figure 2G demonstrates the increased percentage of CD8+ T cells with the engineered IL-2Rβγ receptor called NKTR-214 on Day 5.

NEUROSCIENCE

Acosta-Ruiz A, Gutzeit, VA, Skelly, MJ et *al.* Branched Photoswitchable Tethered Ligands Enable Ultra-Efficient Optical Control and Detection of G Protein-Coupled Receptors *In Vivo*. *Neuron* **105**: 446-463. e13 (2020). DOI: 10.1016/j.neuron.2019.10.036.

Bassil F, Brown H, Pattabhiraman S et *al.* Amyloid-Beta (Aβ) Plaques Promote Seeding and Spreading of Alpha-Synuclein and Tau in a Mouse Model of Lewy Body Disorders with Aβ Pathology. *Neuron* **105**: 260-275.e6 (2020). DOI: 10.1016/j.neuron.2019.10.010.

Bassil F, Meymand ES, Brown HJ et *al.* α-Synuclein modulates tau spreading in mouse brains. *J Exp Med* **218**: e20192193 (2021). DOI: 10.1084/jem.20192193.

Bentsen MA, Rausch DM, Mirzadeh Z et *al.* Transcriptomic analysis links diverse hypothalamic cell types to fibroblast growth factor 1-induced sustained diabetes remission. *Nat Commun* **11**: 4458 (2020). DOI: 10.1038/s41467-020-17720-5.

Engström Ruud L , Pereira MMA, de Solis AJ et *al.* NPY mediates the rapid feeding and glucose metabolism regulatory functions of AgRP neurons. *Nat Commun* **11**: 442 (2020). DOI: 10.1038/s41467-020-14291-3.

He Z, McBride JD, Xu H et *al.* Transmission of tauopathy strains is independent of their isoform composition. *Nat Commun* **11**: 7 (2020). DOI: 10.1038/s41467-019-13787-x.

Jais A, Paeger L, Sotelo-Hitschfeld T et *al.* PNOC^{ARC} Neurons Promote Hyperphagia and Obesity upon High-Fat-Diet Feeding. *Neuron* **106**:1009-1025.e10, (2020). DOI: 10.1016/j.neuron.2020.03.022.

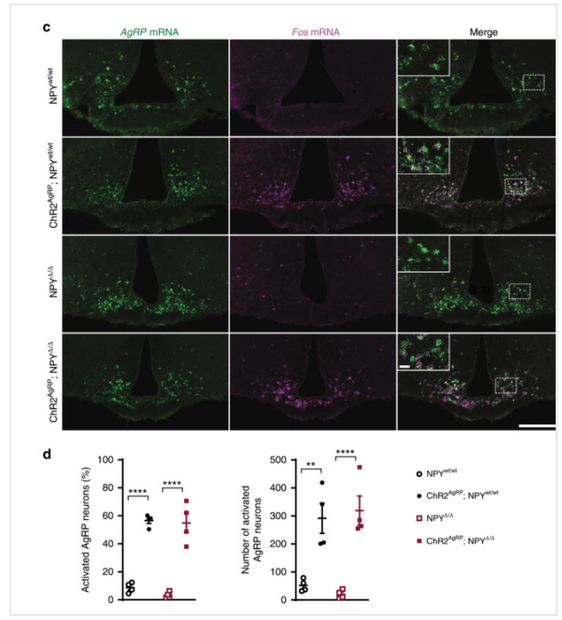
NPY MEDIATES THE RAPID FEEDING AND GLUCOSE METABOLISM REGULATORY FUNCTIONS OF AGRP NEURONS

Authors: LE Ruud, MMA Pereira, AJ de Solis, H Fenselau, J Brüning

Source: https://www.nature.com/articles/s41467-020-14291-3/figures/1

Modification: Cropped

Science: AgRP neurons integrate many signals regarding the energy state of mice and regulate feeding behaviors. Linda Ruud and colleagues elucidate the contribution of neuropeptide Y (NPY) signaling through AgRP neurons in this publication. They show that deficiency of NPY is sufficient to nearly eliminate the immediate feeding behavior associated with AgRP neurons as well as the glucose regulatory function of these neurons. Re-expression of NPY is sufficient to restore both glucose homeostasis and an increase in eating behavior.



Technology: Quantification of RNAscope assays with HALO in Figure 1C-D shown above on NPY-deficient mice hypothalamus tissue. HALO identifies nuclei based on DAPI signal, defines a cytoplasmic ring that expands the nucleus, and detects RNAscope probes based on a combination of parameters. In this experiment, Ruud and colleagues stimulate with light *in vivo* and then examine AgRP signaling. They find that NPY deficiency does not impact the ChR2-dependent activation of AgRP neurons upon laser illumination.

ONCOLOGY

Collet L, Ghurburrun E, Meyers N et *al.* *Kras* and *Lkb1* mutations synergistically induce intraductal papillary mucinous neoplasm derived from pancreatic duct cells. *Gut* **69**: 704-714 (2020). DOI: 10.1136/gutjnl-2018-318059.

Domchek SM, Postel-Vinay S, Im SA, et *al.* Olaparib and durvalumab in patients with germline BRCA-mutated metastatic breast cancer (MEDIOLA): an open-label, multicentre, phase 1/2, basket study. *Lancet Oncol* **21**: 1155-1164 (2020). DOI: 10.1016/S1470-2045(20)30324-7.

Fisher MJ, Shih CS, Rhodes SD et *al.* Cabozantinib for neurofibromatosis type 1–related plexiform neurofibromas: a phase 2 trial. *Nat Med* **27**: 165–173 (2021). DOI: 10.1038/s41591-020-01193-6.

Holmes AB, Corinaldesi C, Shen Q et *al.* Single-cell analysis of germinal-center B cells informs on lymphoma cell of origin and outcome. *J Exp Med* **217**: e20200483 (2020). DOI: 10.1084/jem.20200483.

Hussain A, Voisin V, Poon S, et *al.* Distinct fibroblast functional states drive clinical outcomes in ovarian cancer and are regulated by TCF21. *J Exp Med.* **217**: e20191094 (2020). DOI: 10.1084/jem.20191094.

Jiang H, Liu X, Knolhoff BL, et *al.* Development of resistance to FAK inhibition in pancreatic cancer is linked to stromal depletion. *Gut* **69**: 122-132 (2020). DOI: 10.1136/gutjnl-2018-317424.

Krishnan MS, Rajan KDA, Park J et *al.* Genomic Analysis of Vascular Invasion in HCC Reveals Molecular Drivers and Predictive Biomarkers. *Hepatology* **3**: 10.1002/hep.31614 (2020). DOI: 10.1002/hep.31614.

Najumudeen AK, Ceteci F, Fey SK et *al.* The amino acid transporter SLC7A5 is required for efficient growth of KRAS-mutant colorectal cancer. *Nat Genet* **53**: 16–26 (2021). DOI: 10.1038/s41588-020-00753-3.

Neeb A, Herranz N, Arce-Gallego S. et *al.* Advanced Prostate Cancer with ATM Loss: PARP and ATR Inhibitors. *Eur Urol.* **79**: 200-211 (2020). DOI: 10.1016/j.eururo.2020.10.029.

Park JS, Burckhardt CJ, Lazcano R et *al.* Mechanical regulation of glycolysis via cytoskeleton architecture. *Nature* **578**: 621–626 (2020). DOI: 10.1038/s41586-020-1998-1.

Puca L, Gavyert K, Sailer V et *al.* Delta-like protein 3 expression and therapeutic targeting in neuroendocrine prostate cancer. *Sci Transl Med* **11**: eaav0891 (2019). DOI: 10.1126/scitranslmed.aav0891.

Siersbæk R, Scabia V, Nagarajan S, et *al.* IL6/STAT3 Signaling Hijacks Estrogen Receptor α Enhancers to Drive Breast Cancer Metastasis. *Cancer Cell* **38**: 412-423.e9 (2020). DOI: 10.1016/j.ccell.2020.06.007.

Wang C, Vegna S, Jin H et *al.* Inducing and exploiting vulnerabilities for the treatment of liver cancer. *Nature* **574**: 268–272 (2019). DOI: 10.1038/s41586-019-1607-3.

TGFβ-BLOCKADE UNCOVERS STROMAL PLASTICITY IN TUMORS BY REVEALING THE EXISTENCE OF A SUBSET OF INTERFERON-LICENSED FIBROBLASTS

Authors: AL Grauel, B Nguyen, D Ruddy, T Laszewski, S Schwartz, J Chan, J Chen, M Piquet, M Pelletier, Z Yan, ND Kirkpatrick, J Wu, A deWeck, M Riester, M Hims, FC Geyer, J Wagner, K MacIsaac, J Deeds, R Diwanji, P Jayaraman, Yenyen Yu, Q Simmons, S Weng, A Raza, B Minie, M Dostalek, P Chikkegowda, V Ruda, O Iartchouk, N Chen, R Thierry, J Zhou, I Pruteanu-Malinici, C Fabre, JA Engelman, G Dranoff, V Cremasco

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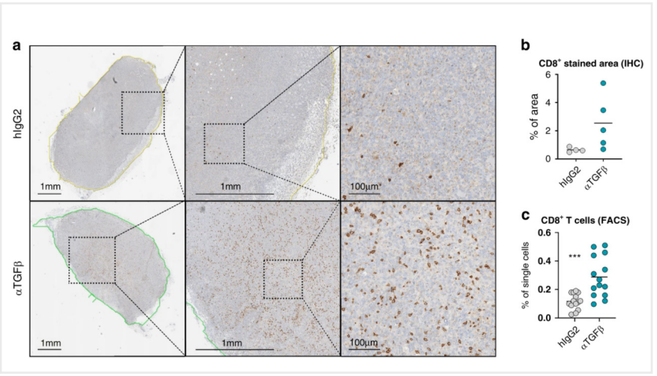

Science: Grauel and colleagues set out to better understand the role of cancer associated fibroblasts (CAFs) in the tumor microenvironment (TME) and found four phenotypically and functionally distinct types of fibroblasts in the TME. Further, they find that TGFβ blockade is associated with a population of CAFs with an immune-permissive microenvironment thus providing a novel pathway to target in cancer. Ongoing clinical trials in patients with malignancies are now being treated with TGFβ neutralizing antibodies in addition to anti-PD1 antibodies.

Technology: The Area Quantification module of HALO was used to quantify CD8a IHC assays comparing tumors from isotype and anti-TGFβ-treated mice as shown in Figure 6a above. They found a substantial difference in the infiltration of cytotoxic T cells between these populations. While a lack of cytotoxic T cells was found in the isotype-treated tumors, extensive CD8+ T-cell infiltration was seen in mice treated with the TGFβ neutralizing antibodies.

MITOGENIC AND PROGENITOR GENE PROGRAMMES IN SINGLE PILOCYTIC ASTROCYTOMA CELLS

Authors: ZJ Reitman, BR Paolella, G Bergthold, K Pelton, S Becker, R Jones, CE Sinai, H Malkin, Y Huang, L Grimmet, ZT Herbert, Y Sun, JL Weatherbee, JA Alberta, JF Daley, O Rozenblatt-Rosen, AL Condurat, K Qian, P Khadka, RA Segal, D Haas-Kogan, MG Filbin, ML Suva, A Regev, CD Stiles, MW Kieran, L Goumnerova, KL Ligon, AK Shalek, P Bandopadhayay, R Beroukhim

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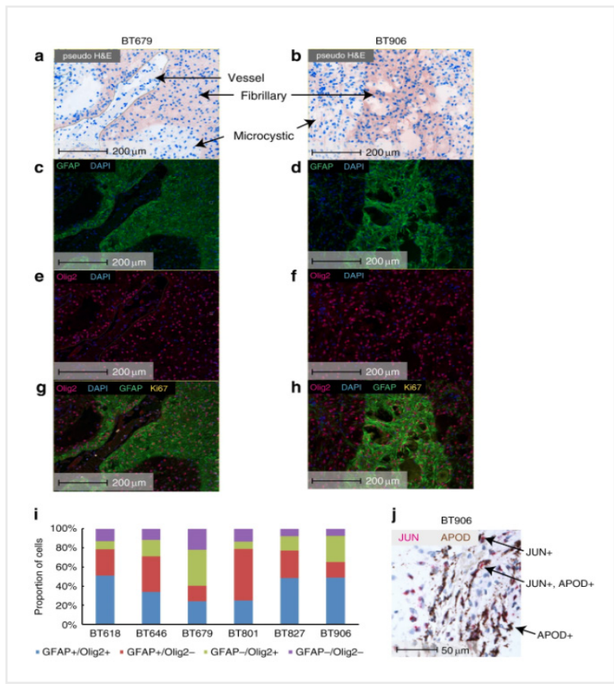

Science: Zachary Reitman and colleagues investigate gene expression signatures associated with a common childhood brain tumor called pilocytic astrocytoma (PA) and identify a cellular developmental process. Further they identified gene expression patterns that differentiate low and high-grade tumors.

Technology: In Figure 6 shown above, immunofluorescence assays were used to validate single cell RNA-seq results. Expression of PA genes identified from the single cell RNA-seq results were examined and corroborated the scRNA-seq analysis. Images were acquired at 20x, and twenty fields of view were analyzed in HALO. Thresholds for positivity were defined, cellular phenotypes were defined and quantified.

OTHER

Cook Sangar ML, Girard EJ, Hopping G *et al.* A potent peptide-steroid conjugate accumulates in cartilage and reverses arthritis without evidence of systemic corticosteroid exposure. *Sci Transl Med* **12**: eaay1041(2020). DOI: 10.1126/scitranslmed.aay1041.

Kim DH, Beckett HD, Nagpal V *et al.* Calpain 9 as a therapeutic target in TGFβ-induced mesenchymal transition and fibrosis. *Sci Transl Med* **11**: 1-15 (2019). DOI: 10.1126/scitranslmed.aau2814.

Stapleton LM, Lucian HJ, Grosskopf AK, *et al.* Dynamic Hydrogels for Prevention of Post-Operative Peritoneal Adhesions. *Adv Ther* **4**: 2000242 (2021). DOI: 10.1002/adtp.202000242.

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