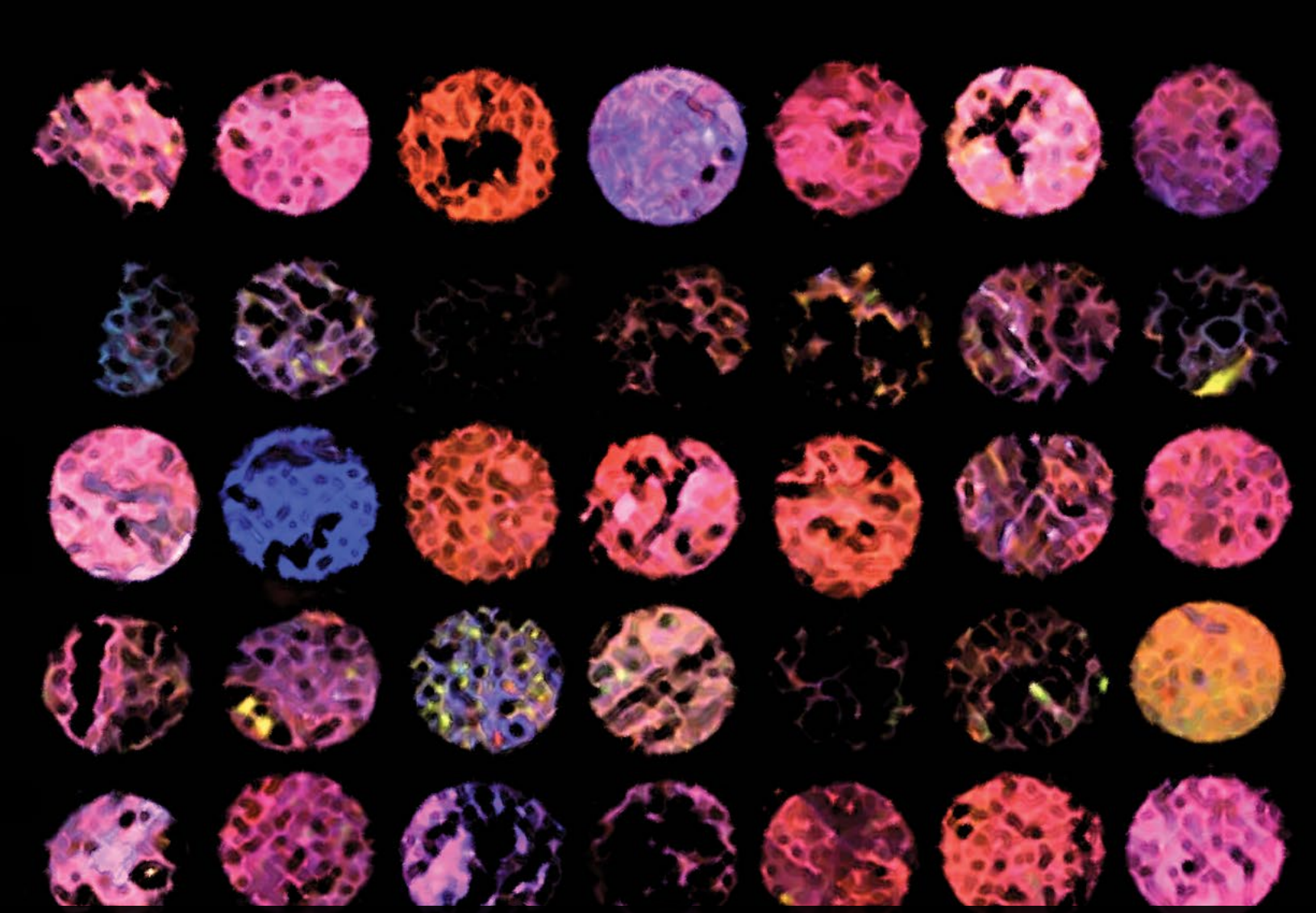

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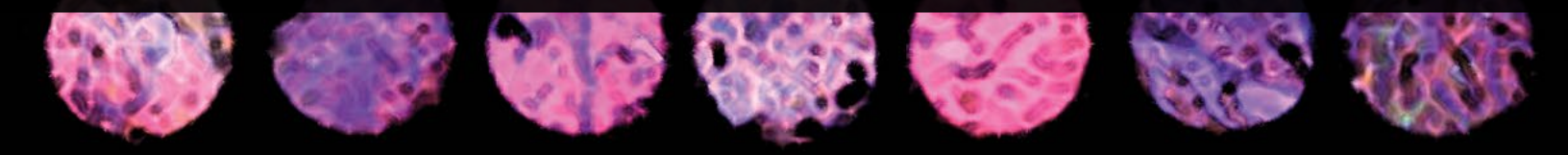

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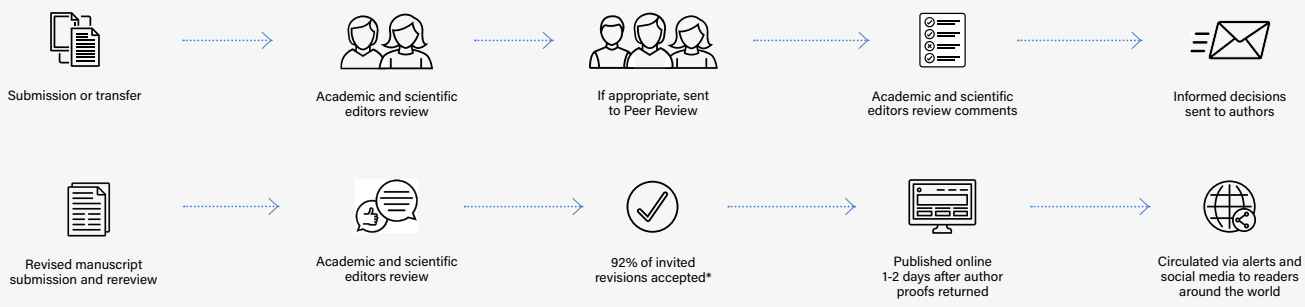

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Since its inception in 1896, the *Journal of Experimental Medicine* (*JEM*) has published articles covering a broad array of disease-related topics, including cancer biology, tumor immunity and immunotherapy. In the last few years, we have made a concerted effort to reemphasize our interest in offering an attractive option for publishing high-quality cancer-related studies in a prestigious journal with broad visibility.

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Brochure articles: Ben Short, PhD, Christina Szalinski, PhD, and Dennis Tartaglia

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On the cover: Multiplexed fluorescence immunohistochemistry of a human lung cancer tissue array, in which tumor tissue and adjacent tissue from the same patient are compared. Image © Yan et al., 2019. Source: Yan, D., J. Wang, H. Sun, A. Zamani, H. Zhang, W. Chen, A. Tang, Q. Ruan, X. Yang, Y. H. Chen, X. Wan. 2020. TIPE2 specifies the functional polarization of myeloid-derived suppressor cells during tumorigenesis. *J Exp Med* 217:e20182005. https://doi.org/10.1084/jem.20182005

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SECRETED SPLICE VARIANTS MEDIATE RESISTANCE TO ANTI-PD-L1 THERAPY

Non-small cell lung cancers can acquire resistance to immunotherapy by producing soluble versions of PD-L1 that can act as decoys and prevent immune checkpoint blockade

Many tumor cells evade the immune system by expressing high levels of the transmembrane protein PD-L1, which binds to its receptor, PD-1, on the surface of cytotoxic T cells and activates an immune checkpoint that inhibits T cell function. Therapeutic antibodies that prevent this checkpoint by binding to PD-1 or PD-L1 have proven to be beneficial treatments for a variety of cancers, from melanoma to non-small cell lung cancer (NSCLC).

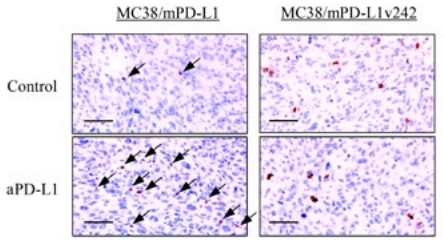
"However, the incidence of acquired resistance to PD-1 and PD-L1 blocking antibodies is increasing," says Ryohei Katayama from the Japanese Foundation for Cancer Research in Tokyo. "Several groups have described mechanisms underlying resistance to PD-1 blockade, but the mechanisms surrounding resistance to anti-PD-L1 treatment remain poorly understood."

Katayama's team, including first author Bo Gong, analyzed two NSCLC patients who initially responded to anti-PD-L1 treatment before undergoing a relapse and found that their relapsed tumors expressed splice variants of PD-L1 that lacked the protein's transmembrane domain. These splice variants are therefore secreted from cells, leading to high levels of soluble PD-L1 in the patients' blood and lung fluid. The same variants were also found in another 2 of 15 cancer patients who had acquired resistance to anti-PD-L1 therapy.

"We hypothesized that the secreted variants act as decoys that attenuate the neutralizing activity of anti-PD-L1 antibodies," says Gong. Indeed, the researchers found that soluble PD-L1 was able to compete for anti-PD-L1 antibodies, preventing them from binding to cell surface PD-L1 and reactivating T cells in vitro.

To test the effects of soluble PD-L1 in vivo, Katayama and colleagues injected mice with murine cancer cells expressing a secreted PD-L1 splice variant and found that the tumors formed by these cells were more resistant to anti-PD-L1 therapy. In fact, the researchers found, only 1% of cells need to express soluble PD-L1 for the tumor to be resistant to PD-L1 blockade. However, the tumors remained susceptible to anti-PD-1 antibodies, suggesting that these could be used as an alternative treatment for patients resistant to anti-PD-L1 therapy.

"Taken together, our findings suggest that the presence of soluble PD-L1 splicing variants or the level of soluble PD-L1 in plasma or pleural effusion may work as a biomarker to predict a patient's response to PD-L1 blockade therapy and that anti PD-1 antibody treatment could be a therapeutic option to overcome soluble PD-L1 variant–induced resistance," Katayama says.



Immune checkpoint blockade with anti-PD-L1 antibodies (bottom row) causes cytotoxic T cells expressing granzyme B (arrows) to accumulate in tumors overexpressing full-length membrane-bound PD-L1 (left). But tumors overexpressing soluble PD-L1 are resistant to anti-PD-L1 treatment (right).
Credit: Gong et al., 2019

RESEARCHER DETAILS

Ryohei Katayama
Chief, Division of Experimental Chemotherapy; Cancer Chemotherapy Center; Japanese Foundation for Cancer Research;
ryohei.katayama@jfcr.or.jp

Bo Gong
Postdoctoral researcher; Division of Experimental Chemotherapy; Cancer Chemotherapy Center; Japanese Foundation for Cancer Research

ORIGINAL PAPER

Gong, B., K. Kiyotani, S. Sakata, S. Nagano, S. Kumehara, S. Baba, B. Besse, N. Yanagitani, L. Friboulet, M. Nishio, K. Takeuchi, H. Kawamoto, N. Fujita, and R. Katayama. 2019. Secreted PD-L1 variants mediate resistance to PD-L1 blockade therapy in non–small cell lung cancer. *J. Exp. Med.* 216:982–1000.
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HETEROTYPIC SPHEROIDS DRIVE OVARIAN CANCER METASTASIS

Fibroblasts and ascitic cancer cells form metastatic units that promote the dissemination of tumor cells throughout the abdominal cavity

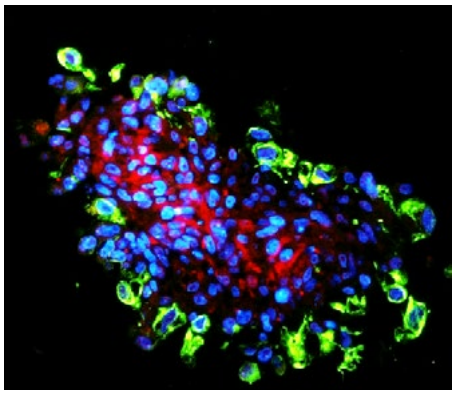
High-grade serous ovarian cancer (HGSOC)—the most aggressive form of ovarian cancer—is characterized by the early and rapid dissemination of cancer cells to other sites within the abdomen. Cancer cells that escape from the primary tumor are thought to form spheroids in the ascitic fluid that accumulates in HGSOC patients. These spheroids can then attach to the peritoneal membrane that lines the abdominal cavity and invade the underlying extracellular matrix to form secondary, metastatic tumors.

"Given the proposed function of spheroids during ovarian cancer metastasis, we wanted to investigate the processes by which ascitic tumor cells (ATCs) assemble into spheroids and execute peritoneal dissemination," says Qinglei Gao from Tongji Medical College, Huazhong University of Science and Technology in Wuhan, China.

Gao and colleagues, including co-first author Zongyuan Yang, found that ATCs from HGSOC patients tend to form heterotypic spheroids with cancer-associated fibroblasts (CAFs) present in the ascites. These CAFs protect the cancer cells from apoptosis and facilitate their invasion of the peritoneum, eventually forming the stroma of the newly formed metastases. "Due to their inherent malignant potential and contribution to peritoneal dissemination, we termed these CAF-containing heterospheroid structures metastatic units," Gao says. "Intriguingly, stromal fibroblasts and the resultant heterospheroids are rarely found in low-grade serous ovarian cancer, which might explain its reduced tendency for dissemination."

Gao's team determined that ATCs from HGSOC patients express high amounts of integrin α5, a cell adhesion molecule whose levels correlate with poor patient outcomes. The researchers found that integrin α5 mediates the association of ATCs with fibroblasts during spheroid formation and that EGF secreted from these fibroblasts subsequently helps to sustain integrin α5 expression. Inhibiting this signaling pathway with a neutralizing anti-EGF antibody impaired spheroid formation and reduced peritoneal tumor burden in mice injected with both ATCs and CAFs.

Gao et al.'s results suggest that targeting CAFs could prevent metastasis in HGSOC patients. The researchers found that early administration of imatinib, a tyrosine kinase inhibitor that can eliminate CAFs by blocking PDGF signaling, reduced tumor burden and improved survival in a mouse model of metastatic ovarian cancer. This treatment was even more effective when combined with liposome clodronate to additionally eliminate tumor-associated macrophages, which have also been reported to promote spheroid formation in ovarian cancer.



Heterotypic spheroids consisting of epithelial cancer cells (green) surrounding a core of fibroblasts (red) form in the ascites of HGSOC patients. These spheroids act as metastatic units that facilitate the dissemination of ovarian cancer cells throughout the abdominal cavity.
Credit: Gao et al., 2019

"Together, our results suggest that early targeting of stromal CAFs to destroy metastatic units could be a new therapeutic strategy to limit HGSOC progression," Gao says.

RESEARCHER DETAILS

Qinglei Gao
Professor; Tongji Medical College; Huazhong University of Science and Technology;
qingleigao@hotmail.com

Zongyuan Yang
Attending doctor, Assistant research fellow; Tongji Medical College; Huazhong University of Science and Technology

ORIGINAL PAPER

Gao, Q., Z. Yang, S. Xu, X. Li, X. Yang, P. Jin, Y. Liu, X. Zhou, T. Zhang, C. Gong, X. Wei, D. Liu, C. Sun, G. Chen, J. Hu, L. Meng, J. Zhou, K. Sawada, R. Fruscio, T.W. Grunt, J. Wischhusen, V.M. Vargas-Hernández, B. Pothuri, and R.L. Coleman. 2019. Heterotypic CAF-tumor spheroids promote early peritoneal metastasis of ovarian cancer. *J. Exp. Med.* 216: 688–703.
https://doi.org/10.1084/jem.20180765

EPIGENETIC PROTEIN COULD BE NEW THERAPEUTIC TARGET IN ACUTE MYELOID LEUKEMIA

The histone methyltransferase EZH2 can delay the development of AML but then maintains tumor growth once the disease is established

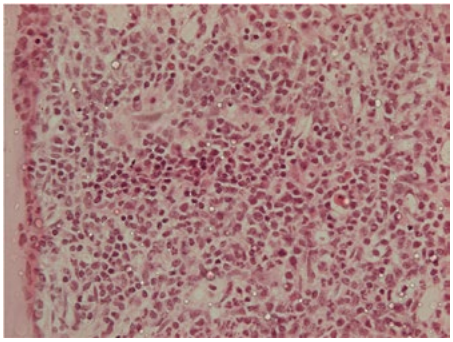
The epigenetic protein EZH2 is the core component of the PRC2 complex that transcriptionally represses hundreds of genes by di- and trimethylating histone H3 on lysine 27. Increases in EZH2 activity are thought to promote the development of a variety of human tumors, including breast and prostate cancers, and several clinical trials are currently investigating whether drugs that prevent EZH2 from modifying histones could be used as cancer treatments.

Whether EZH2 also promotes the development of blood cancers is unclear, however. Some evidence suggests that the epigenetic protein may actually prevent myeloid malignancies, including acute myeloid leukemia (AML), a particularly aggressive blood cancer that is expected to kill over 10,000 people in the United States alone this year.

A team of researchers led by Professor Brian Huntly at the Cambridge Institute for Medical Research, UK, found that upon acquisition of specific AML driver mutations, mice lacking Ezh2 developed the disease much faster than expected, indicating that the protein does indeed delay the development of AML. However, deleting the *Ezh2* gene (or chemically inhibiting the Ezh2 protein) after AML had fully developed and established itself in the mice, dramatically disrupted tumor growth and significantly prolonged the animals' survival. Inhibiting EZH2 also prevented the growth of AML cells isolated from patients.

Huntly and colleagues, including co-first authors Faisal Basheer and George Giotopoulos, found that inhibiting EZH2 has conflicting effects on the development and maintenance of AML because the protein regulates almost completely different sets of genes at early and late stages of the disease. For example, during the initial stages of AML, loss of Ezh2 causes cells to increase production of a transcription factor called Plag1 that accelerates the development of leukemia. But inhibiting Ezh2 at later stages of AML has no effect on Plag1 levels.

"Our findings uncover novel and dramatically opposing functions of EZH2 during AML that appear dependent upon the phase of disease, with EZH2 functioning as a tumor suppressor in AML induction and as a facilitator of disease in established AML," Huntly says. "To our knowledge, this is the first description of an epigenetic regulator having both tumor-suppressive and oncogenic function in different phases of the same cancer. In addition, our work validates EZH2 as a therapeutic target with the potential to treat several different subtypes of AML."



Loss of EZH2 accelerates the formation of leukemic cells in the bone marrow of mice expressing the oncogenic fusion protein AML1-ETO9a.
Credit: Basheer et al., 2019

RESEARCHER DETAILS

Faisal Basheer
PhD student/Clinical Research Fellow
Cambridge Stem cell Institute

George Giotopoulos
Research Associate
Cambridge Institute for Medical Research

Brian J.P. Huntly
Professor of Leukaemia Stem Cell Biology
Cambridge Stem cell Institute
bjph2@cam.ac.uk

ORIGINAL PAPER

Basheer, F., G. Giotopoulos, E. Meduri, H. Yun, M. Mazan, D. Sasca, P. Gallipoli, L. Marando, M. Gozdecka, R. Asby, O. Sheppard, M. Dudek, L. Bullinger, H. Döhner, R. Dillon, S. Freeman, O. Ottmann, A. Burnett, N. Russell, E. Papaemmanuil, R. Hills, P. Campbell, G.S. Vassiliou, and B.J.P. Huntly. 2019. Contrasting requirements during disease evolution identify EZH2 as a therapeutic target in AML. *J. Exp. Med.* 216: 966–981.
https://doi.org/10.1084/jem.20181276

IL-1β CONDITIONING IMPROVES CELL-BASED IMMUNOTHERAPY

Approach could expand use of adoptive cell therapy for epithelial cancers

Adoptive cell therapy (ACT), the infusion of immune system cells to eliminate cancer, has shown great promise. Infusion of tumor-infiltrating lymphocytes and genetically modified autologous T cells can lead to long-term remission in some cancers. Although great progress has been made with these therapeutic approaches, they remain ineffective for a majority of patients with common epithelial cancers.

Because IL-1β has been shown to enhance the protective value of weak vaccines, Ping-Hsien Lee, Nicholas P. Restifo, and colleagues from the National Cancer Institute and University of Pennsylvania set out to determine whether administration of IL-1β can improve the efficacy of adoptively transferred T cells in causing tumor regression. Their study involved mice with large, established B16 melanoma tumors treated with IL-1β, in conjunction with ACT.

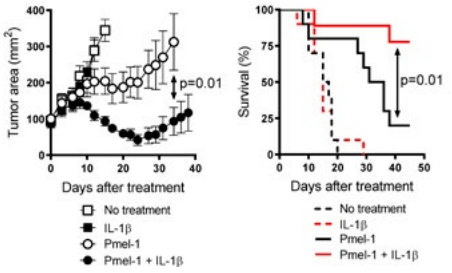
The researchers inoculated mice with B16 melanoma cells engineered to overexpress the altered gp100 peptide (B16-mhgp100), and allowed them to grow for 10 days. They then administered whole-body irradiation to mice with demonstrated B16-mhgp100 tumors, and infused premelanosome protein-1 (Pmel-1) $CD8^+$ T cells and injections of vehicle or IL-1β. Tumor-draining lymph nodes and the tumors were excised after the last injection.

"We found that IL-1β treatment increased Pmel-1 cell numbers only in the tumor, but enhanced granzyme B expression in both the tumor-draining lymph nodes and the tumor," notes first author Ping-Hsien Lee. This indicated that IL-1β triggered the T cells to differentiate and become more effector like."

In an earlier phase of this study, researchers administered a lower dose/shorter duration of IL-1β than had been used in a previous study in which high dosage IL-1β strongly enhanced $CD8^+$ T cell responses but caused inflammation and subsequent morbidity and mortality in the mice. In the current study, the lower dosage IL-1β infusion retained the ability to increase the numbers and function of adoptively transferred T cells without causing severe systemic inflammation.

Administration of lower dose IL-1β showed no impact on growth of existing B16-mhgp100 tumors or mouse survival. However, it improved the ability of adoptively transferred Pmel-1 cells to induce tumor regression and significantly prolonged the survival of tumor-bearing mice. The researchers did not observe any mortality that could be ascribed to the treatment.

Further analysis revealed that IL-1β acts both directly and indirectly to increase the efficacy of ACT. IL-1β induced a broad array of gene expression in $CD8^+$ T cells—an effector like gene signature—and improved local T cell accumulation in peripheral tissues by enhancing tissue trafficking and survival of the adoptively transferred cells. Host cells were found to play an essential role in mediating IL-1β-driven granzyme B expression in T cells through a mechanism involving key T cell growth and survival cytokines IL-2 and IL-15.



Administration of IL-1β enhances the antitumor function of Pmel-1 cells to mediate tumor regression and prolong mouse survival.
Credit: Lee et al., 2019

"We demonstrated that administration of IL-1β can increase the efficacy of adoptively transferred T cells in order to mediate tumor regression," says senior author Nicholas Restifo. "The use of IL-1β to condition the host environment must be continued in the clinic in order to enhance the ability of adoptively transferred T cells to eradicate tumors."

The authors would like to recognize William E. Paul, MD, who initiated this work. Dr. Paul died from cancer in 2015 before the study was completed.

RESEARCHER DETAILS

Ping-Hsien Lee
Staff Scientist
Children's National Health System
leepinghsien@gmail.com

Nick Restifo
Executive Vice President, Research
Lyell Immunopharma Inc.
drnickrestifo@gmail.com

ORIGINAL PAPER

Lee, P.-H., T. N. Yamamoto, D. Gurusamy, M. Sukumar, Z. Yu, J. Hu-Li, T. Kawabe, A. Gangaplara, R. J. Kishton, A. N. Henning, S. K. Vodnala, R. N. Germain, W. E. Paul, and N. P. Restifo. 2019. Host conditioning with IL-1β improves the antitumor function of adoptively transferred T cells. *J. Exp. Med.* 216: 2619–2634.
https://doi.org/10.1084/jem.20181218

OBSERVING CAR T CELL ACTIVITY IN VIVO

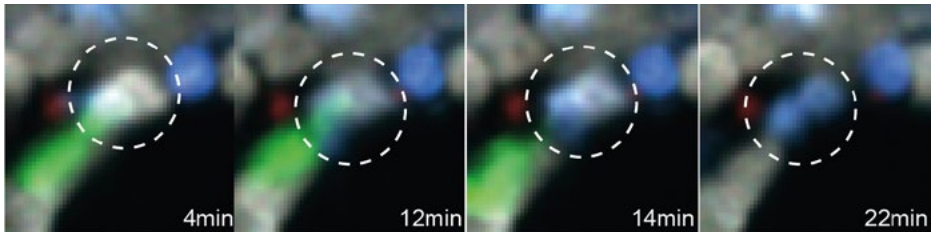
Single-cell intravital imaging reveals that genetically engineered T cells can induce tumor regression by direct killing of cancer cells

Genetically engineered chimeric antigen receptor (CAR) T cells that target a protein called CD19, which is found on many lymphoma and leukemia cells, are an FDA-approved treatment for several B cell malignancies, including non-Hodgkin's lymphoma and acute lymphoblastic leukemia.

Some patients fail to respond to anti-CD19 CAR T cells, however, while others, after an initial improvement, relapse and develop therapy-resistant tumors that no longer express CD19. Philippe Bousso and colleagues at the Institut Pasteur believe that the key to improving treatment is to learn more about how CAR T cells work. For instance, it was unclear if CAR T cells kill the cancer cells themselves or whether they induce other immune cells to attack the tumor.

Bousso and colleagues, including co-first authors Marine Cazaux and Capucine Grandjean, tracked the activity of anti-CD19 CAR T cells injected into mice with B cell lymphoma. Using intravital two-photon imaging, the researchers were able to see individual CAR T cells killing lymphoma cells in the animals' bone marrow. Some CAR T cells appeared to be more active than others, but, in most cases, lymphoma cells died within minutes of coming into direct contact with a CAR T cell.

"Computer simulations based on our experimental data supported the idea that CAR T cells rely on their direct cytotoxic activity rather than on the recruitment and activation of other cells to eliminate the bulk of the B cell lymphoma," Bousso says.



Intravital two-photon imaging of the bone marrow of a mouse with B cell lymphoma shows a CAR T cell (green) come into contact with a live tumor cell (gray) and quickly kill it (causing it to turn blue).
Credit: Cazaux et al., 2019

The simulations also suggested that treatment outcome may depend on the CAR T cells' ability to infiltrate the bone marrow. One reason why CAR T cells may fail to infiltrate the bone marrow is if they encounter lymphoma cells, or healthy B cells that also express CD19, circulating in the blood. Bousso and colleagues found that CAR T cells can aggregate with these circulating cells and become trapped in the lungs, preventing them from reaching the bone marrow. Reducing these encounters—for example, by temporarily reducing the number of circulating B cells—enhanced the ability of CAR T cells to infiltrate the bone marrow and kill tumor cells, prolonging the survival of mice with B cell lymphoma.

"Purging both circulating tumor and normal B cells prior to CAR T cell transfer may therefore offer a clinical benefit by improving CAR T cell engraftment and persistence," Bousso says.

Still, the researchers found that tumor relapse and the emergence of tumors lacking CD19 occur in the bone marrow, rather than in other organs affected by B cell lymphoma, such as the lymph nodes. This appears to be because CAR T cells are not as active in these other organs, reducing the incentive for tumor cells to lose the CD19 protein.

RESEARCHER DETAILS

Capucine L. Grandjean
Postdoctoral researcher
Institut Pasteur

Philippe Bousso
Group Leader
Institut Pasteur
philippe.bousso@pasteur.fr

Marine Cazaux
PhD student
Institut Pasteur

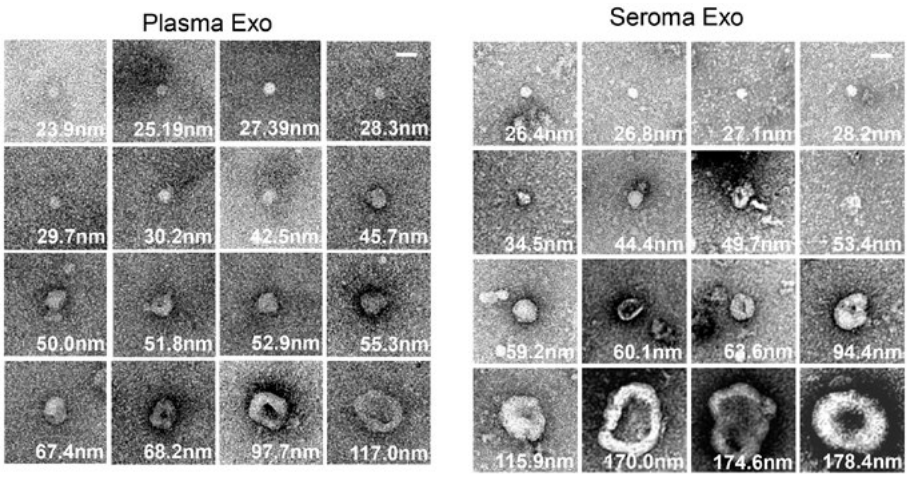
ORIGINAL PAPER

Cazaux, M., C.L. Grandjean, F. Lemaître, Z. Garcia, R.J. Beck, I. Milo, J. Postat, J.B. Beltman, E.J. Cheadle, and P. Bousso. 2019. Single-cell imaging of CAR T cell activity in vivo reveals extensive functional and anatomical heterogeneity. *J. Exp. Med.* 216:1038–1049.
https://doi.org/10.1084/jem.20182375

LYMPHATIC EXUDATE IS A RICH SOURCE OF MELANOMA BIOMARKERS

Two studies reveal that cancer-associated proteins, miRNAs, and DNA sequences can all be detected in extracellular vesicles present in the lymph of metastatic melanoma patients

Biomarkers allow physicians to monitor the progression of tumors, follow their response to treatment, and predict the likelihood of recurrence. Liquid biopsies of patient blood samples are particularly advantageous because they are minimally invasive and may facilitate the early detection of disease progression/relapse by monitoring the presence of circulating biomolecules released from the tumor microenvironment. This may be particularly important for melanoma, an aggressive cancer that is prone to metastasis but, as of yet, there is no reliable blood test for melanoma occurrence or recurrence.



Extracellular vesicles in lymphatic exudate (left) are more numerous and, on average, larger than their counterparts in plasma (right).
Credit: García-Silva et al., 2019

Two independent research teams—one led by Melody Swartz and Maria Broggi at the University of Chicago, the other by Héctor Peinado and Susana García-Silva at the Spanish National Cancer Research Center in Madrid—wondered whether lymphatic fluid could be an alternative source of melanoma biomarkers available for liquid biopsy. Molecules secreted in the tumor microenvironment drain into lymphatic vessels and are transported to lymph nodes before entering the general circulation, suggesting that biomarkers may be present at higher levels in lymph than in the blood. Moreover, many of the factors released from tumors are thought to target specific tissues, such as the lymph nodes, where they can establish a premetastatic niche for tumor cells to spread to.

"We therefore hypothesized that lymph carries important tumor-derived factors that reflect the tumor microenvironment and may allow patient stratification or help predict response to therapy," Swartz says. Swartz and colleagues collected lymphatic exudate from metastatic melanoma patients undergoing lymphadenectomy and found that numerous melanoma-associated proteins were enriched in lymph compared with blood plasma. The biomarkers lactate dehydrogenase and S100B, for example, were highly abundant in lymphatic exudate but undetectable in plasma samples from the same patients. Lymphatic exudate also contained higher concentrations of multiple miRNAs that are secreted by melanomas and are thought to promote metastasis.

Many proteins and nucleic acids secreted from tumors are carried within exosomes or other types of extracellular vesicle. "We

RESEARCHER DETAILS

Susana García-Silva
Staff Scientist
Spanish National Cancer Research Center (CNIO)

Héctor Peinado
Group Leader
Spanish National Cancer Research Center (CNIO)
hpeinado@cnio.es

ORIGINAL PAPER

García-Silva, S., A. Benito-Martín, S. Sánchez-Redondo, A. Hernández-Barranco, P. Ximénez-Embún, L. Nogués, M.S. Mazariegos, K. Brinkmann, A.A. López, L. Meyer, C. Rodríguez, C. García-Martín, J. Boskovic, R. León, C. Montero, M. Robledo, L. Santambrogio, M.S. Brady, A. Szumera-Ciećkiewicz, I. Kalinowska, J. Skog, M. Noerholm, J. Muñoz, P.L. Ortiz-Romero, Y. Ruano, J.L. Rodríguez-Peralto, P. Rutkowski, and H. Peinado. 2019. Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and *BRAF*^{V600E} mutation. *J. Exp. Med.* 216: 1061–1070.
https://doi.org/10.1084/jem.20181522

decided to characterize extracellular vesicles in lymphatic exudate obtained after lymphadenectomy in metastatic melanoma patients," says Peinado.

Peinado and colleagues found that lymphatic exudate contains many more extracellular vesicles than plasma. The protein content of these vesicles was also different, with the researchers identifying more than 700 proteins that were more abundant in lymph-derived exosomes than in plasma-derived vesicles. In addition, proteomic profiling revealed that lymph-derived exosomes are highly similar to exosomes collected from human melanoma cell lines in vitro.

Swartz and colleagues also saw an increased number of extracellular vesicles in the lymphatic exudate of melanoma patients and they determined that most of the cancer-associated proteins and miRNAs in lymph were contained within these carriers. Furthermore, the researchers were able to compare the lymphatic exudates of patients at different stages of disease and identify unique protein signatures for early and advanced metastatic spread.

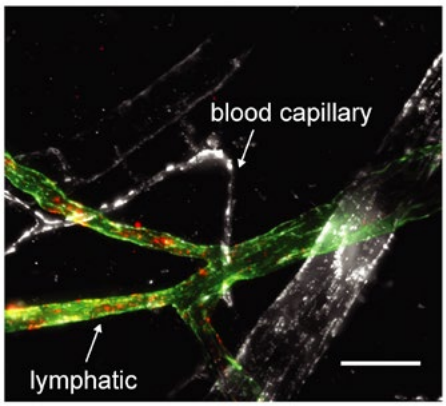
"In patients with no evidence of metastatic spread beyond the sentinel lymph node, the proteomic profile of extracellular vesicles from lymphatic exudate was associated with pathways involved in cellular movement, vascularization, extravasation, and adhesion, which correlate with early stages of metastasis," Broggi explains. "In contrast, the proteomic profile of extracellular vesicles isolated from patients with further nodal spread showed up-regulated signaling pathways connected to cell death, proliferation, and cancer, suggestive of more advanced disease."

Peinado and colleagues also saw that proteins involved in numerous melanoma-associated signaling pathways were enriched in lymphatic exudate-derived extracellular vesicles, with proteins of the RAS/RAF/MAPK network particularly enriched in patients with advanced metastatic disease.

The researchers were unable to use these biomarkers to predict patient outcome but they did identify a potential prognostic indicator when they examined the DNA associated with lymphatic exudate extracellular vesicles. Mutations in the V600 codon of the *BRAF* gene are found in 35–50% of human melanomas and increased levels of mutant *BRAF* DNA in cell-free plasma fractions have been shown to correlate with poorer outcomes. Peinado and colleagues were able to identify mutant copies of the *BRAF* gene associated with extracellular vesicles isolated from the lymphatic exudate of melanoma patients.

"Remarkably, among patients diagnosed as $BRAF^{V600E}$ by tissue biopsy, patients that tested positive for this mutation in their lymphatic extracellular vesicles had a median survival time of 146.5 days versus 715 days for patients that tested negative," says García-Silva.

In fact, the presence of $BRAF^{V600E}$ in lymphatic exudate-derived extracellular vesicles predicted patient outcome independently of tumor *BRAF* status. "Thus, detection of *BRAF* mutation in extracellular vesicles obtained through lymphatic drainage after lymphadenectomy may be a novel parameter to identify patients at risk of relapse probably due to the presence of residual disease," Peinado says. "These patients could subsequently benefit from specific adjuvant therapies right after surgery."



Tumor-derived extracellular vesicles (red) are taken up into lymphatic vessels (green) after intradermal injection into mouse ear dermis, demonstrating the role of these vessels in transporting tumor-released factors to the systemic circulation.
Credit: Broggi et al., 2019

Swartz and colleagues also identified a unique cellular signature in the lymphatic exudate of melanoma patients, including increased numbers of memory T cells with tumor-reactive or stem cell–like phenotypes. "Taken together, our data suggest that lymph from cancer patients is a rich source of tumor-derived factors and may provide a highly valuable source for identifying protein, miRNA, extracellular vesicle, and cellular fingerprints of each patient's cancer," says Swartz. "Further analysis on lymph collected from a larger cohort of melanoma patients, incorporating a wider range of disease states, will be required to fully explore its potential for biomarker discovery that could guide the design of personalized therapies."

RESEARCHER DETAILS

Maria A.S. Broggi
Postdoctoral researcher
University of Chicago
(Currently a Lab Head at Novartis Institute of Biomedical Research)
mas.broggi@gmail.com

Melody A. Swartz
William B. Ogden Professor of Molecular Engineering
University of Chicago
melodyswartz@uchicago.edu

ORIGINAL PAPER

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RIG-I AGONIST INDUCES ANTITUMOR IMMUNE RESPONSE

A stem loop RNA that stimulates RIG-I activation delays tumor growth, extends survival, and prevents tumor recurrence

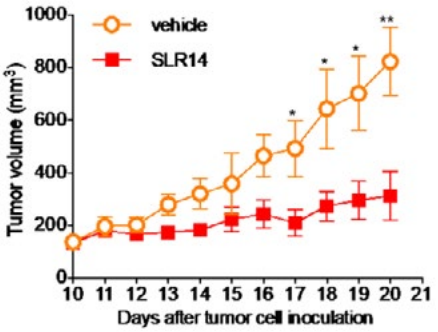
Immunotherapy harnesses the body's own capabilities in combating cancers, and Xiaodong Jiang, Anna Marie Pyle, Akiko Iwasaki, and colleagues at Yale University reveal a new treatment that can be used alone or in combination with existing immunotherapies to shrink tumors. They show that a synthetic RNA molecule, similar to one found in pathogens, can induce antitumor immune responses.

The innate immune system relies on receptors that detect pathogen-associated molecules from invading microbes to initiate a protective immune response. A sensor of cytosolic RNA called RIG-I is key in antiviral immune responses. "Our idea was to mimic a viral infection inside the tumor, tricking the immune system into thinking there is an infection and getting rid of it," Iwasaki said. Recent evidence has shown that RIG-I activation in cancer cells by RNA ligands can induce cancer cell apoptosis.

The Yale team designed a small RNA named Stem Loop RNA 14 (SLR14), specifically designed to activate RIG-I. In a melanoma mouse model, the researchers injected SLR14 directly into tumors and observed tumor growth delay and extended mouse survival. Because several mouse cancer models had previously been responsive to treatment with anti-PD1, an immune checkpoint inhibitor, Jiang and colleagues tried anti-PD1 in combination with SLR14, which showed enhanced antitumor effects in both melanoma and colon cancer models.

Using a fluorescent SLR14 injected into tumor cells, the researchers observed where the molecule went and found that SLR14 was taken up by 80% of the CD11b$^+$ myeloid cells in the tumor microenvironment. Transcriptomic analysis of tumors after SLR14 treatment showed many genes associated with immune defense were significantly up-regulated after treatment. The team observed an increase in the number of CD8$^+$ T lymphocytes, NK cells, and CD11b$^+$ cells in the SLR14-treated tumors.

To see how broadly SLR14 treatment could be applied, the team tried another melanoma model, called B16, that is notoriously resistant to immune therapies. SLR14 induced a robust antitumor activity, even in this poorly immunogenic B16 model. In mice with two B16 tumors, injection of SLR14 into one inhibited the growth of the other, leading the team to also test a metastasis model in which SLR14 inhibited metastatic tumor growth. In tumor-cured B16 mice, the team re-injected B16 tumor cells, and no tumor growth occurred, suggesting SLR14 triggered the immune system's "memory."



Average tumor volume in a colon cancer mouse model treated with control (vehicle) or SLR14.
Credit: Jiang et al., 2019

"Our results demonstrate that SLR14 is a promising therapeutic RIG-I agonist for cancer treatment, either alone or in combination with existing immunotherapies," Jiang says.

RESEARCHER DETAILS

Xiaodong Jiang
Associate Research Scientist
Yale University

Anna Marie Pyle
Professor
Yale University
anna.pyle@yale.edu

Akiko Iwasaki
Professor
Yale University
akiko.iwasaki@yale.edu

ORIGINAL PAPER

Jiang, X., V. Muthusamy, O. Fedorova, Y. Kong, D.J. Kim, M. Bosenberg, A.M. Pyle, and A. Iwasaki. Intratumoral delivery of RIG-I agonist SLR14 induces robust antitumor responses. 2019. *J. Exp Med.* 216:2854–2868.
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CREATINE POWERS ANTITUMOR IMMUNITY

Study suggests that creatine supplementation can improve T cell–based cancer immunotherapies

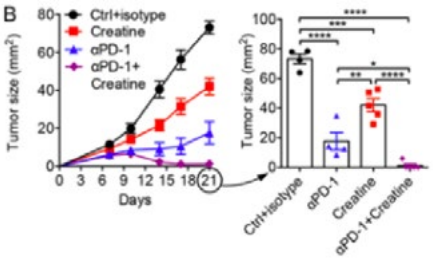
Creatine is a popular supplement for bodybuilders and athletes and new research shows that it is also crucial in combating cancer. Stefano Di Biase, Xiaoya Ma, Lili Yang, and colleagues at University of California, Los Angeles (UCLA) studied the nutrient usage of tumor-infiltrating immune cells and found that creatine plays a key role that could be harnessed to improve T cell-based cancer immunotherapies.

T cells are a central responder in the body's fight against cancer, and have recently been employed for immunotherapies, but "in the tumor microenvironment, T cells face the special challenge of competing with fast-growing tumor cells for metabolic fuel such as glucose, amino acids, and lipids, which can be limiting," Di Biase says. To study the metabolic regulators of anti-tumor immune cells, the researchers analyzed gene expression in a mouse melanoma model. Comparing immune cells from the tumor site with those outside of tumor site (i.e., from spleen) revealed a sharp increase in expression of a gene called *CrT* (or *Slc6a8*), which controls creatine uptake.

Though *CrT* has a well-established role in muscles and the brain, its functions outside those tissues were largely known. The team found that tumor growth was accelerated in *CrT*-knockout mice, which led them to generate transgenic mice with CrT knocked out in tumor-specific CD8 T cells. These *CrT*-knockout CD8 T cells were less able to rein in tumor growth and showed a reduction in almost all aspects of T cell activation, including cell proliferation and production of effector cytokines such as IL-2 and IFN-γ.

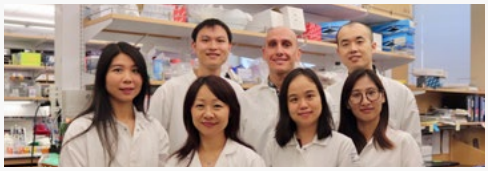
The team showed that giving supplemental creatine to mice, at doses comparable to those recommended for athletes, significantly suppressed tumor growth in multiple tumor models. The combination of creatine supplementation with a PD-1/PD-L1 blockade treatment showed enhanced tumor suppression effects, compared to either treatment alone. "Because oral creatine supplements have been broadly utilized by bodybuilders and athletes for the past three decades, existing data suggest they are likely safe when taken at appropriate doses," said Yang. "This could provide a clear and expedient path forward for the use of creatine supplementation to enhance existing cancer immunotherapies."

Their results, combined with what is known about creatine-dependent cells, led the researchers to propose that CD8 T cells work like hybrid cars, using two distinct energy sources. Their "fuel engine" converts glucose, amino acids, and lipids into ATP, while creatinine serves as a "molecular battery" to store bioenergy and buffer ATP levels to support anti-tumor activity. This helps the T cells to perform in the metabolically stressful tumor microenvironment. "It is also likely that creatine regulates immune reactions to multiple diseases beyond cancer, such as infections and autoimmune diseases," Di Biase says.



Creatine supplementation in combination with anti–PD-1 (αPD-1) treatment in an MC38 colon cancer model shows reduced tumor growth compared to control or either treatment alone.
Credit: Di Biase et al., 2019

RESEARCHER DETAILS



Pictured: Lili Yang, Stefano Di Biase, Jessie Xiaoya Ma, Shirley Xi Wang, Victor Jiaji Yu, Ryan Yuchen Wang, and Alice Yang Zhou

Stefano Di Biase
Postdoctoral Fellow
University of California, Los Angeles

Xiaoya Ma
Postdoctoral Fellow
University of California, Los Angeles

Lili Yang
Assistant Professor
University of California, Los Angeles
liliyang@ucla.edu

ORIGINAL PAPER

Di Biase, S., X. Ma, X. Wang, J. Yu, Y. Wang, D.J. Smith, Y. Zhou, Z. Li, Y.J. Kim, N. Clarke, A. To, and L. Yang. Creatine uptake regulates CD8 T cell antitumor immunity. 2019. *J. Exp Med.* 216:2869–2882.
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PREDICTING CANCER NEOANTIGENS THAT DRIVE CD8 T CELL RESPONSES

Mutation position proves to be an important determinant of immunogenic neoantigens, which play a role in individualized cancer treatment

Studies have shown that tumor-specific mutations can generate neoantigens that stimulate T cell responses against cancer cells, which has prompted researchers to develop immunotherapies such as cancer vaccines that target neoantigens. However, researchers have found that only a small fraction of predicted neoantigens are immunogenic, and the accurate selection of immunogenic neoantigens poses a challenge.

Addressing this, a team from Genentech, led by senior scientists Aude-Hélène Capietto, Lélia Delamarre, and Suchit Jhunjhunwala discovered that mutation position is a significant determinant of immunogenic neoantigens.

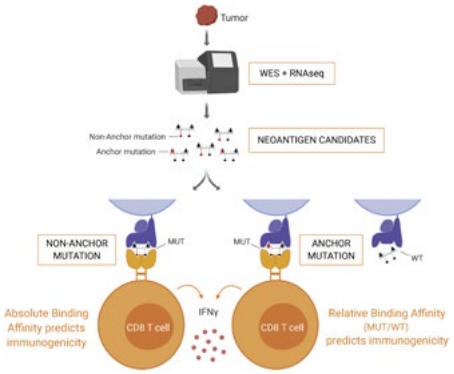
To learn this, the group systematically assessed the immunogenicity of peptides containing single amino acid mutations in mice. They selected 416 mutated neoepitopes from four mouse tumor cell lines based on their predicted binding affinity to MHC-I, a frequent consideration in prioritizing neoantigens. Immunogenicity was tested by vaccinating mice with synthetic long peptides containing the mutations. Only neoantigens from 40 mutations induced CD8 T cell responses.

Next, the team assessed the immunogenic potential of predicted neoepitopes of 39 of 40 antigenic mutations. They were surprised to find that in 43.6% of cases, neoantigen-specific CD8 cells generated by vaccination did not recognize or poorly recognized the best predicted neoepitope candidate.

"We've confirmed that a large majority of candidate neoantigens, as predicted by binding affinity alone, are not antigenic with this vaccine," Capietto says. "Further, our results show that peptide binding ability to MHC-I is not sufficient to predict immunogenicity."

Testing the cross-reactivity of neoantigenic-specific CD8 cells with the wild-type counterpart of the predicted epitope showed a high rate of cross-reactivity. Recognition of the wild-type counterpart by the neoantigen-specific T cell receptor, however, was generally weaker and independent of predicted binding affinities.

The researchers hypothesized that the position of the mutation may play a role in predicting immunogenicity of mutated neoepitopes. Based on their predicted role in binding to MHC-I, mutations were classified as affecting either anchor or non-anchor positions, and binding affinity was analyzed for the mutant and its wild-type counterpart. For anchor mutations, the relative affinity of the mutant and wild-type epitopes is a strong predictor of immunogenicity. For non-anchor mutations, however, the absolute affinity of the mutant neoepitope better predicts immunogenicity. Importantly, these results were validated with human data.



Mutation position plays a key role in predicting the immunogenicity of neoepitopes.
Credit: Capietto et al., 2019

Analysis of mouse vaccination data showed that ranking to prioritize immunogenic candidates can be improved by taking into account the anchor versus non-anchor position of the mutation.

"We were happy to see predictive properties of neoantigens in the mouse data were also predictive in human data," Delamarre says. "This approach may soon be used to improve the prioritization of neoepitope candidates and thus lead to more effective immunotherapies, including personalized cancer vaccination approaches."

RESEARCHER DETAILS

Aude-Hélène Capietto, PhD
Senior Scientific Researcher,
Cancer Immunology
Genentech
capietta@gene.com

Lélia Delamarre, PhD
Senior Scientist,
Cancer Immunology
Genentech
delamarre.lelia@gene.com

ORIGINAL PAPER

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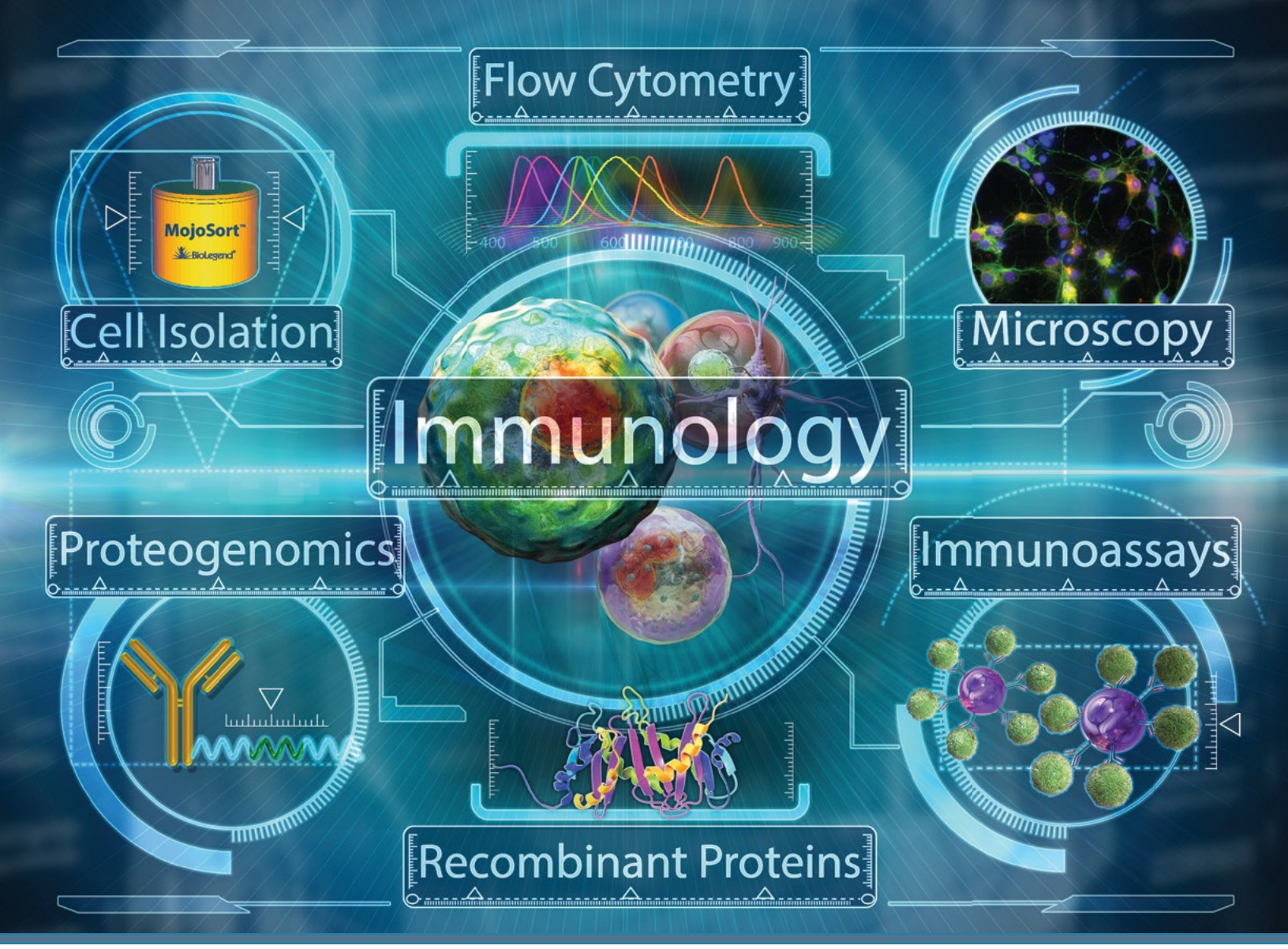

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