SPECIAL EDITION: Cancer

ScienceTranslationalMedicine.org

Science Translational Medicine

AAAS

TACONIC

MORE PREDICTIVE

MORE HUMAN

SETTING THE STANDARD IN GENETICALLY ENGINEERED & PRECISION RESEARCH RODENT MODELS

Genetically Engineered Models & Services
Precision Research Models
Integrated Custom Model Generation & Breeding

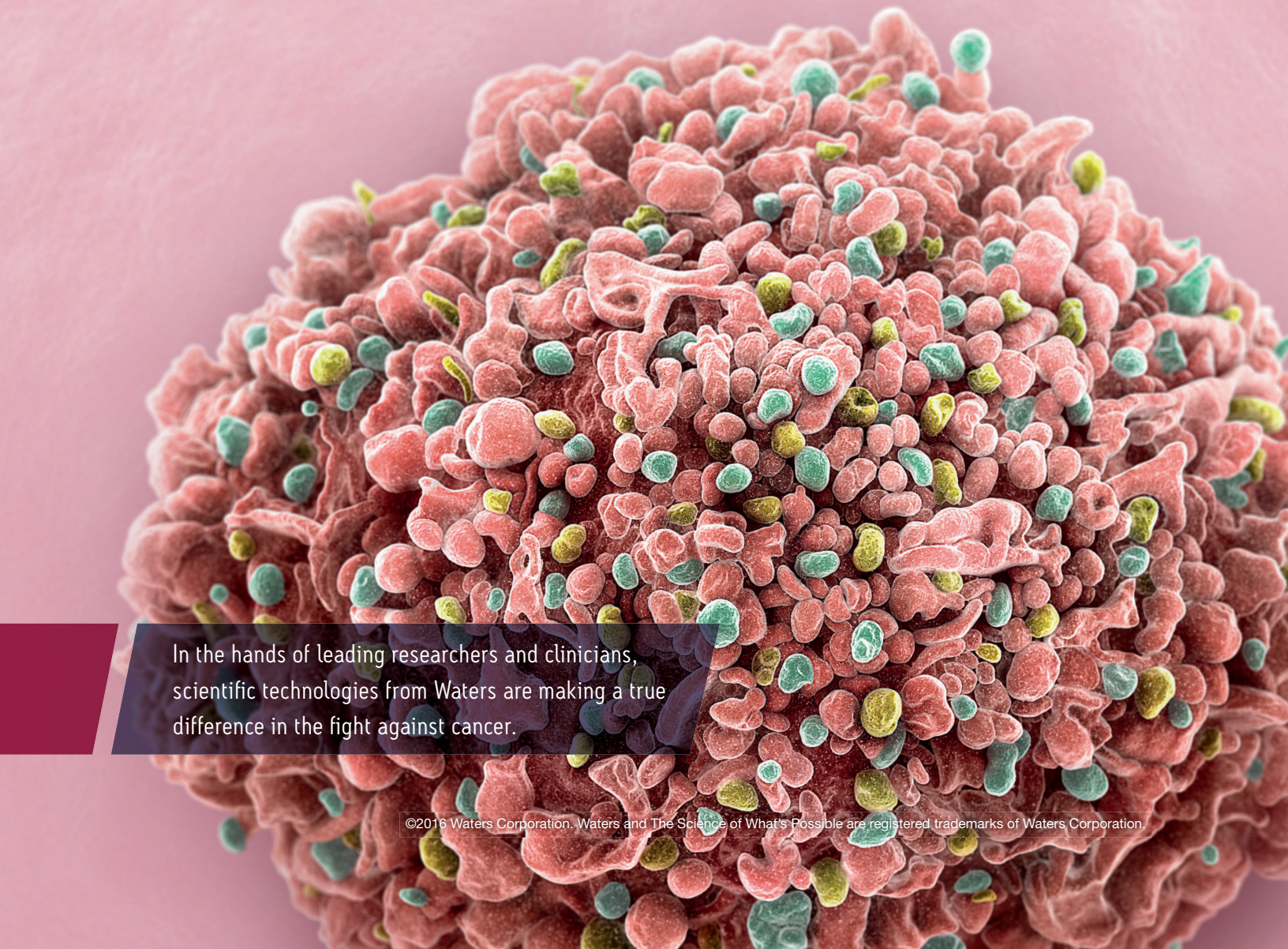
TACONIC.COM | US: +1 888 822-6642 | EU: +45 70 23 04 052 | INFO@TACONIC.COM

IT'S NOT TECHNOLOGY
FOR TECHNOLOGY'S SAKE.
IT'S FOR HUMANITY'S SAKE.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Where scientific discovery meets new hope, that's where you'll find Waters. Continuing to create innovative tools to enable breakthrough discoveries in the understanding of complex diseases such as cancer. And, working with partners across the health sciences spectrum to bring our technologies and systems closer to the patient – combining research, diagnostics and real-time analyses for the benefit of all of us. To learn more about our science in the service of humanity, visit waters.com/healthsciences

PHARMACEUTICAL ▪ HEALTH SCIENCES ▪ FOOD ▪ ENVIRONMENTAL ▪ CHEMICAL MATERIALS



In the hands of leading researchers and clinicians, scientific technologies from Waters are making a true difference in the fight against cancer.

SPECIAL EDITION: Cancer

Select research published in *Science Translational Medicine*

Science Translational Medicine is the leading journal of high-impact, peer reviewed research at the intersection of biomedical sciences and clinical applications. Part of the *Science* family of journals, it is published online weekly and showcases exciting translational research that matters most for human health. Topics include immunology, regenerative medicine, cancer, infectious disease, drug discovery, neurology, genomic medicine, bioengineering, and other interdisciplinary areas. Highlights are full-length research papers and topical editorials, reviews, and commentaries.
[Credit: C. Bickel/*Science Translational Medicine*]

IN THIS BOOKLET

Learn more and submit your research today: ScienceTranslationalMedicine.org

ion torrent

Because each tumor sample matters

All it takes is a few slides to uncover meaningful variants from FFPE tumor samples

Every sample is unique and tells a story. One that you are driven to understand, even when there is very limited material available. The Ion Torrent™ Oncomine™ next-generation sequencing assays utilize proven Ion AmpliSeq™ technology with low sample input requirements to help you accurately identify variants from more tumor samples and fine needle aspirates. Obtain the answers you need for your research to prevail. Pursue. Pioneer. Prevail.

To learn more about Oncomine Assays for oncology research, visit us at **thermofisher.com/oncomine-assays**

ThermoFisher SCIENTIFIC

Science Translational Medicine | AAAS
INTEGRATING SCIENCE, ENGINEERING, AND MEDICINE

January 2016
Washington, DC

Dear Colleagues,

In this booklet on Cancer, the Editors of *Science Translational Medicine* have assembled some of the best examples of translational research in cancer that have appeared in our pages.

An adaptive and resilient disease, cancer constantly challenges modern medicine to innovate. Here we showcase the latest approaches to diagnose, treat, and prognosticate in the field of human oncology.

Research and policy articles come together to highlight emerging ideas in:

- Cancer genomics
- Drug delivery and imaging
- Drug resistance
- Immunotherapy
- Circulating tumor markers
- Cancer stem cells
- Diagnostics and prognostics
- Cancer vaccines

We thank our sponsors Affymetrix, Inc., Taconic Biosciences, Inc., Thermo Fisher Scientific Inc., and Waters Corporation for their support, and we hope you enjoy this collection.

Sincerely,

Katrina L. Kelner, Ph.D.
Editor

CANCER

Cancer and the gut microbiota: An unexpected link

Laurence Zitvogel,[1,2]*† Lorenzo Galluzzi,[1,3,4,5]* Sophie Viaud,[1,2] Marie Vétizou,[1,2] Romain Daillère,[1,2] Miriam Merad,[6] Guido Kroemer[3,4,5,7,8]

Changes in the interactions among the gut microbiota, intestinal epithelium, and host immune system are associated with many diseases, including cancer. We discuss how environmental factors influence this cross-talk during oncogenesis and tumor progression and how manipulations of the gut microbiota might improve the clinical activity of anticancer agents.

One hundred trillion organisms (mainly bacteria) collectively referred to as the gut microbiota colonize the human intestine. Reflecting a notable degree of coevolution, the gut microbiota thrives in mutually advantageous equilibrium with the host (eubiosis). The intestine offers a protected, warm, and nutrient-rich microenvironment to resident microbes, while the gut microbiota assists humans in the digestion of complex carbohydrates, provides them with non-nutrient essential factors, and occupies ecological niches that might otherwise be colonized by pathogenic microorganisms (*1*). The immune system tolerates the normal gut microbiota while ensuring immunosurveillance against invading pathogens. Moreover, accumulating evidence indicates that the proper development of both intestinal and extraintestinal components of the immune system requires the gut microbiota (*2*). In this Perspective, we discuss how disequilibria in the intimate relationship between the host and intestinal bacteria (dysbiosis) affect oncogenesis, tumor progression, and response to cancer therapy and how the gut microbiota may be manipulated for therapeutic purposes. A detailed description of the intestinal immune system is beyond the scope of this article and can be found in (*2*).

[1]Gustave Roussy Comprehensive Cancer Center, F-94805 Villejuif, France. [2]INSERM, U1015, ClCBT507, F-94805 Villejuif, France. [3]Equipe 11 Labellisée par la Ligue Nationale Contre le Cancer, Centre de Recherche des Cordeliers, F-75006 Paris, France. [4]Université Paris Descartes/Paris V, Sorbonne Paris Cité, F-75006 Paris, France. [5]INSERM, U1138, F-75006 Paris, France. [6]Department of Oncological Sciences, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA. [7]Pôle de Biologie, Hôpital Européen Georges Pompidou F-75015, AP-HP, Paris, France. [8]Metabolomics and Cell Biology Platforms, Gustave Roussy Comprehensive Cancer Center, F-94805 Villejuif, France.
*These authors contributed equally to this work.
†Corresponding author. E-mail: laurence.zitvogel@gustaveroussy.fr

DYSBIOSIS AND CARCINOGENESIS

Dysbiosis can be caused not only by pathogenic organisms and passenger commensals but also by aging and environmental factors such as antibiotics, xenobiotics, smoking, hormones, and dietary cues (*1*); these are also well-established risk factors for the development of intestinal or extraintestinal neoplasms. In addition, genetic defects that affect epithelial, myeloid, or lymphoid components of the intestinal immune system favor dysbiosis because they promote inflammatory states, such as Crohn's disease, that increase the host's risk for neoplastic transformation (*3*). Thus, several factors that favor carcinogenesis also promote dysbiosis.

Epidemiological studies that link intraabdominal infections, the use of antibiotics, or both to an increased incidence of colorectal cancer (*4*) underscore the clinical importance of the association between dysbiosis and intestinal carcinogenesis. In fact, the gut microbiota affects colorectal carcinogenesis by various mechanisms. Abrogating or specifically altering the composition of the gut microbiota influences the incidence and progression of colorectal carcinoma in both genetic and carcinogen-induced models of tumorigenesis (*5*–*7*). Moreover, several by-products of the gut microbiota directly target intestinal epithelial cells (IECs) and either mediate oncogenic effects (as reported for hydrogen sulfide and the *Bacteroides fragilis* toxin) or suppress tumorigenesis (as demonstrated for short-chain fatty acids) (*8*).

Intestinal bugs participate in more than just colorectal carcinogenesis. Experimental alterations of the gut microbiota also influence the incidence and progression of extraintestinal cancers, including breast and hepatocellular carcinoma, presumably through inflammatory and metabolic circuitries (*9*, *10*). These results are compatible with the findings of epidemiological studies that reveal an association between dysbiosis, its consequences or determinants (in particular the overuse of antibiotics), and an increased incidence of extracolonic neoplasms, including breast carcinoma (*11*, *12*). These findings may reflect the systemic distribution of bacteria and their by-products in the course of inflammatory responses that compromise the integrity of the intestinal barrier (*9*).

Thus, the gut microbiota influences oncogenesis and tumor progression both locally and systemically. Although inflammatory and metabolic cues support this phenomenon, additional, hitherto uncharacterized mechanisms can contribute to the ability of dysbiosis to promote carcinogenesis (Fig. 1).

RELATIONSHIP STATUS: IT'S COMPLICATED

During cancer therapy, the gut microbiota and antineoplastic agents interact in a bidirectional fashion. On the one hand, several interventions currently used for the management of neoplastic diseases exert cytotoxic effects on intestinal bacteria, de facto promoting dysbiosis (*13*). Thus, radiation therapy, allogeneic stem cell transplantation, and several chemotherapeutic agents such as irinotecan (a topoisomerase I inhibitor licensed for the treatment of colorectal carcinoma) and 5-fluorouracil (a nucleoside analog used against several carcinomas) can be toxic for the gut microbiota—and hence alter its composition—either directly or by activating an immune response (*14*–*16*). Moreover, these (and other) therapeutic interventions exert unwarranted side effects on the intestinal barrier (table S1). On the other hand, accumulating evidence indicates that the gut microbiota influences both the therapeutic activity and the side effects of anticancer agents, via pharmacodynamic (*17*, *18*) and immunological mechanisms (*19*, *20*) (Fig. 2).

Pharmacodynamic effects. By virtue of their abundance and pronounced metabolic activity, intestinal bacteria can determine the bioavailability and biological effects, be they warranted (efficacy) or not (toxicity), of ingested xenobiotics. This has been demonstrated for several drugs, including irinotecan (*17*, *18*). The dose-limiting diarrhea associated with irinotecan has been attributed to the ability of the gut microbiota to reactivate the drug locally (*17*). Moreover,

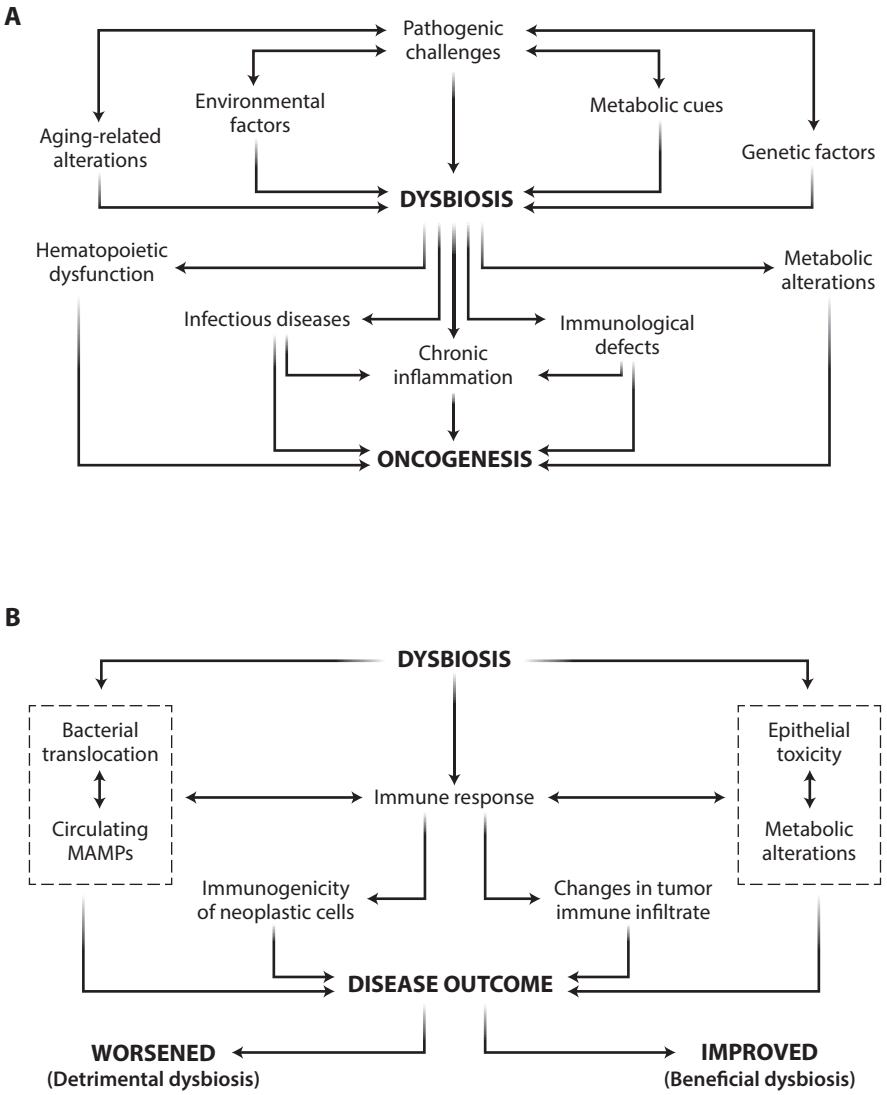


Fig. 1. Links between dysbiosis and cancer. (**A**) Mechanisms by which dysbiosis affects oncogenesis. (**B**) Detrimental and beneficial effects of dysbiosis on disease outcome. MAMP, microbe-associated molecular pattern.

the gastrointestinal toxicity of irinotecan is reduced by the administration of a Chinese herbal medicine (PHY906) that acquires the ability to stimulate the regeneration of intestinal progenitor cells only upon transformation by bacterial β-glucuronidase (which is highly expressed by the gut microbiota) (*18*).

The effects of the gut microbiota on the pharmacodynamics of anticancer agents may not be limited to orally administered molecules (which physically get in contact with intestinal bacteria), but may involve systemic interventions. Indeed, germ-free (GF) mice have been reported to differ from their conventional, pathogen-free counterparts in the expression of a broad panel of hepatic genes involved in xenobiotic metabolism (*21*). Moreover, the gut microbiota may play a critical role in the elicitation of acute graft-versus-host disease (GVHD), a critical obstacle against the clinical success of allogeneic stem cell transplantation. Several reports link dysbiosis (most often characterized by an enrichment in *Enterobacteriaceae spp.*) to overt infections and intestinal GVHD, with a major role for Paneth cell destruction and alterations in the TLR9/MYD88 signaling axis (*15*, *22*). Thus, besides influencing the gastrointestinal side effects of some anticancer interventions, dysbiosis may undermine their therapeutic activity. Conversely, a eubiotic gut microbiota may limit the unwarranted side effects of various antineoplastic agents.

Immunological effects. Accumulating evidence indicates that the gut microbiota also modulates the response of several tumor types to cancer therapy via immunological circuitries, at least in mice (*19*, *20*, *23*). For example, lymphodepleting total body irradiation reportedly promotes the translocation of the gut microbiota or at least some of its components or products across the intestinal epithelium. This not only correlates with increased dendritic cell activation and elevated levels of blood-borne proinflammatory cytokines but also contributes to the ability of irradiation to maximize the efficacy of adoptively transferred $CD8^+$ T lymphocytes (*23*). Accordingly, antibiotic-treated mice, mice injected with a lipopolysaccharide (LPS)–neutralizing antibody, as well as *Cd14*$^{-/-}$ and *Tlr4*$^{-/-}$ mice (which do not respond to LPS normally) are less sensitive to lymphodepleting irradiation than are their control counterparts (*23*).

The injection of cyclophosphamide (an immunostimulatory alkylating agent used against multiple carcinomas) into mice maintained in pathogen-free conditions promotes mucosal injury and translocation of specific Gram-positive bacteria across the intestinal epithelium (*20*). This phenomenon was linked to therapeutically relevant T helper type 1 (T_H1) and T_H17 immune responses in the spleen (*20*). GF and antibiotic-treated tumor-bearing mice, which failed to mount such antibacterial T cell–mediated responses, were more resistant than their control counterparts to the therapeutic effects of cyclophosphamide (*20*). Moreover, the full-blown antineoplastic activity of cyclophosphamide could be restored in antibiotic-treated mice upon the adoptive transfer of T_H17 cells established and propagated in vitro (*20*). However, not all Gram-positive bacteria were able to elicit beneficial T_H17 immune responses in this setting. Rather, specific prokaryotes such as *Parabacteroides distasonis* [which exerts regulatory T (T_{reg}) cell–stimulatory effects] and segmented filamentous bacteria (which trigger conventional T_H17 responses) reduced the beneficial effects of anticancer chemotherapy.

Consistent with these data, a healthy gut microbiota has been shown to contribute to the therapeutic activity of a CpG oligodeoxynucleotide-based immunotherapeutic regimen and platinum derivatives (*19*). The gut microbiota influenced the propensity of CpG oligodeoxynucleotides combined with a monoclonal antibody that neutralizes

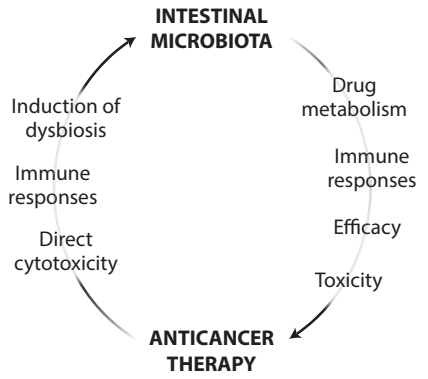


Fig. 2. Links between the gut microbiota and anticancer therapy. Intestinal bacteria interact with chemo-, radio-, and immunotherapeutic anticancer agents in a bidirectional manner.

interleukin-10 receptor α (IL10RA) to elicit a therapeutically relevant, tumor necrosis factor–α (TNF-α)–dependent innate immune response against malignant cells. In addition, a eubiotic gut microbiota was necessary for oxaliplatin (an immunogenic platinum salt approved for use in colorectal cancer patients) to promote tumor infiltration by myeloid cells that mediated antineoplastic effects by producing reactive oxygen species (ROS) (*19*). In line with this notion, the chemotherapy-impairing effects of antibiotics could be mimicked by the *Cybb*$^{-/-}$ genotype (corresponding to the lack of a ROS-generating enzyme) as well as by the systemic administration of antioxidants (*19*). Mice lacking *Myd88* or *Tlr4* (encoding critical components of the machinery sensing microbe-associated molecular patterns) were also more resistant to oxaliplatin-based chemotherapy than were their wild-type counterparts (*19*). Thus, the full-blown therapeutic activity of oxaliplatin involves the detection of components of the gut microbiota by the immune system, allowing for the generation of tumor-infiltrating myeloid cells with antineoplastic activity.

Altogether, these observations indicate that anticancer therapy can promote two functionally opposite types of dysbiosis: detrimental dysbiosis, which limits the therapeutic efficacy or increases the toxicity of treatment, and beneficial dysbiosis, which is required for, or at least markedly improves, its clinical activity (Fig. 1). This suggests that the pharmacological manipulation of the gut microbiota holds great promise as an adjuvant to improve the therapeutic index of anticancer therapy.

MANIPULATING THE MICROBIOTA FOR CANCER THERAPY

At least hypothetically, four distinct measures can be used to alter the effects of the gut microbiota on anticancer therapy: (i) antibiotics, chemicals with a preferential cytotoxicity for one or more bacterial species; (ii) probiotics, living bacteria or other microorganisms that, when administered in adequate amounts, confer a health benefit; (iii) prebiotics, nondigestible compounds that stimulate the growth and/or functions of specific components of the gut microbiota; and (iv) postbiotics, nonviable products of the gut microbiota that exert biological activities in the host.

Using common antibiotics (which often target multiple types of Gram-positive or Gram-negative bacteria) to cause a state of dysbiosis that supports, rather than counteracts, the efficacy of chemotherapeutic agents may not be feasible because of specificity issues. However, it may be possible to use antibiotics to reverse a previously established state of detrimental dysbiosis (*24*). Recent data indicate that bacteriocins, proteinaceous antibiotics produced by some bacterial strains, may be harnessed to specifically deplete one or a few components of the gut microbiota for therapeutic purposes (*1*). Moreover, specific chemicals may be successfully used to limit the negative impact of the gut microbiota on the pharmacodynamics of specific chemotherapeutics. As a proof of principle, a potent inhibitor of bacterial (but not mammalian) β-glucuronidase has been shown to protect mice from the intestinal side effects of irinotecan, widening its therapeutic window (*17*).

Probiotics have been extensively tested in animal tumor models for their ability to prevent (mostly intestinal) carcinogenesis, with promising results (*25*, *26*). Moreover, genetically modified probiotics have been successfully used as vectors for the delivery of tumor-associated antigens, immunostimulatory molecules, or enzymes that limit the toxicity of conventional chemotherapy, at least in animal models (*27*). Some of these approaches, notably anticancer vaccines based on live, attenuated variants of *Listeria monocytogenes* or *Salmonella enterica*, are currently being tested for their safety and ability to elicit therapeutically relevant immune responses in cancer patients (*28*), reflecting a considerable progress in the academic and industrial development of vaccines harnessing mucosal immunity (*29*).

Thus far, epidemiological studies have been unable to firmly establish whether probiotics can reduce the risk of developing colorectal carcinoma in specific patient populations (*26*). Similarly, clinical data on the use of probiotics as a means to limit the gastrointestinal toxicity of radiation therapy and some chemotherapeutics are insufficient to draw a firm conclusion on their actual benefits (*30*). Although prebiotics (such as inulin or oligofructose) and postbiotics (such as butyrate) have attracted attention as potential means of preventing colorectal cancer, the ability of these agents to widen the therapeutic window of chemotherapy remains poorly explored (*31*).

In view of the recent findings showing that specific alterations in the gut microbiota are instrumental, rather than detrimental, to the efficacy of anticancer chemotherapy, it is tempting to speculate that the clinical profile of at least some chemotherapeutics can be improved by combinatorial interventions relying on one or more antibiotics, prebiotics, probiotics, and/or postbiotics. This hypothesis urgently awaits experimental confirmation.

Accumulating evidence demonstrates that intestinal bacteria influence oncogenesis, tumor progression, and response to therapy. Thus, selectively manipulating the gut microbiota may represent a feasible means to (i) limit the incidence of specific tumors in the general population and/or (ii) improve the activity of various anticancer agents (*32*). Although the first possibility has been investigated in several models of oncogenesis with promising results, the actual oncopreventive effects of anti-, pre-, pro-, and postbiotics in humans remain to be established. Conversely, selectively manipulating the composition of the gut microbiota as a gateway to optimal responses to chemo-, radio-, or immunotherapy in the clinic is a relatively new concept, and additional studies are required to understand the clinical value of such an approach. In this context, the limited selectivity of most conventional antibiotics and the elevated interindividual heterogeneity of the gut microbiota may constitute major obstacles. Highly specific antimicrobials such as bacteriocins and the development of new technologies allowing for the rapid in-depth characterization of the gut microbiota on a personalized basis may circumvent these issues, at least in part. Modulating the gut microbiota may constitute a viable strategy for improving the clinical efficacy of anticancer chemo-, radio-, and immunotherapy.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/271/271ps1/DC1

Table S1. Links between the gastrointestinal side effects of common anticancer regimens and the gut microbiota.

REFERENCES AND NOTES

1. R. F. Schwabe, C. Jobin, The microbiome and cancer. *Nat. Rev. Cancer* **13**, 800–812 (2013).
2. C. L. Maynard, C. O. Elson, R. D. Hatton, C. T. Weaver, Reciprocal interactions of the intestinal microbiota and immune system. *Nature* **489**, 231–241 (2012).
3. N. Kamada, S. U. Seo, G. Y. Chen, G. Núñez, Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* **13**, 321–335 (2013).
4. J. L. Wang, C. H. Chang, J. W. Lin, L. C. Wu, L. M. Chuang, M. S. Lai, Infection, antibiotic therapy and risk of colorectal cancer: A nationwide nested case-control study in patients with Type 2 diabetes mellitus. *Int. J. Cancer* **135**, 956–967 (2014).
5. M. Bonnet, E. Buc, P. Sauvanet, C. Darcha, D. Dubois, B. Pereira, P. Déchelotte, R. Bonnet, D. Pezet, A. Darfeuille-Michaud, Colonization of the human gut by *E. coli* and colorectal cancer risk. *Clin. Cancer Res.* **20**, 859–867 (2014).
6. J. C. Arthur, E. Perez-Chanona, M. Mühlbauer, S. Tomkovich, J. M. Uronis, T. J. Fan, B. J. Campbell, T. Abujamel, B. Dogan, A. B. Rogers, J. M. Rhodes, A. Stintzi, K. W. Simpson, J. J. Hansen, T. O. Keku, A. A. Fodor, C. Jobin, Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* **338**, 120–123 (2012).
7. Y. Zhan, P. J. Chen, W. D. Sadler, F. Wang, S. Poe, G. Núñez, K. A. Eaton, G. Y. Chen, Gut microbiota protects against gastrointestinal tumorigenesis caused by epithelial injury. *Cancer Res.* **73**, 7199–7210 (2013).
8. P. Louis, G. L. Hold, H. J. Flint, The gut microbiota, bacterial metabolites and colorectal cancer. *Nat. Rev. Microbiol.* **12**, 661–672 (2014).
9. S. Yoshimoto, T. M. Loo, K. Atarashi, H. Kanda, S. Sato, S. Oyadomari, Y. Iwakura, K. Oshima, H. Morita, M. Hattori, K. Honda, Y. Ishikawa, E. Hara, N. Ohtani, Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. *Nature* **499**, 97–101 (2013).
10. D. H. Dapito, A. Mencin, G. Y. Gwak, J. P. Pradere, M. K. Jang, I. Mederacke, J. M. Caviglia, H. Khiabanian, A. Adeyemi, R. Bataller, J. H. Lefkowitch, M. Bower, R. Friedman, R. B. Sartor, R. Rabadan, R. F. Schwabe, Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. *Cancer Cell* **21**, 504–516 (2012).
11. C. Xuan, J. M. Shamonki, A. Chung, M. L. Dinome, M. Chung, P. A. Sieling, D. J. Lee, Microbial dysbiosis is associated with human breast cancer. *PLOS One* **9**, e83744 (2014).
12. C. M. Velicer, S. R. Heckbert, J. W. Lampe, J. D. Potter, C. A. Robertson, S. H. Taplin, Antibiotic use in relation to the risk of breast cancer. *J. Am. Med. Assoc.* **291**, 827–835 (2004).
13. Y. Touchefeu, E. Montassier, K. Nieman, T. Gastinne, G. Potel, S. Bruley des Varannes, F. Le Vacon, M. F. de La Cochetière, Systematic review: The role of the gut microbiota in chemotherapy- or radiation-induced gastrointestinal mucositis—Current evidence and potential clinical applications. *Aliment. Pharmacol. Ther.* **40**, 409–421 (2014).
14. Y. D. Nam, H. J. Kim, J. G. Seo, S. W. Kang, J. W. Bae, Impact of pelvic radiotherapy on gut microbiota of gynecological cancer patients revealed by massive pyrosequencing. *PLOS One* **8**, e82659 (2013).
15. R. R. Jenq, C. Ubeda, Y. Taur, C. C. Menezes, R. Khanin, J. A. Dudakov, C. Liu, M. L. West, N. V. Singer, M. J. Equinda, A. Gobourne, L. Lipuma, L. F. Young, O. M. Smith, A. Ghosh, A. M. Hanash, J. D. Goldberg, K. Aoyama, B. R. Blazar, E. G. Pamer, M. R. van den Brink, Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J. Exp. Med.* **209**, 903–911 (2012).
16. I. Von Bültzingslöwen, I. Adlerberth, A. E. Wold, G. Dahlén, M. Jontell, Oral and intestinal microflora in 5-fluorouracil treated rats, translocation to cervical and mesenteric lymph nodes and effects of probiotic bacteria. *Oral Microbiol. Immunol.* **18**, 278–284 (2003).
17. B. D. Wallace, H. Wang, K. T. Lane, J. E. Scott, J. Orans, J. S. Koo, M. Venkatesh, C. Jobin, L. A. Yeh, S. Mani, M. R. Redinbo, Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* **330**, 831–835 (2010).
18. W. Lam, S. Bussom, F. Guan, Z. Jiang, W. Zhang, E. A. Gullen, S. H. Liu, Y. C. Cheng, The four-herb Chinese medicine PHY906 reduces chemotherapy-induced gastrointestinal toxicity. *Sci. Transl. Med.* **2**, 45ra59 (2010).
19. N. Iida, A. Dzutsev, C. A. Stewart, L. Smith, N. Bouladoux, R. A. Weingarten, D. A. Molina, R. Salcedo, T. Back, S. Cramer, R. M. Dai, H. Kiu, M. Cardone, S. Naik, A. K. Patri, E. Wang, F. M. Marincola, K. M. Frank, Y. Belkaid, G. Trinchieri, R. S. Goldszmid, Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013).
20. S. Viaud, F. Saccheri, G. Mignot, T. Yamazaki, R. Daillère, D. Hannani, D. P. Enot, C. Pfirschke, C. Engblom, M. J. Pittet, A. Schlitzer, F. Ginhoux, L. Apetoh, E. Chachaty, P. L. Woerther, G. Eberl, M. Bérard, C. Ecobichon, D. Clermont, C. Bizet, V. Gaboriau-Routhiau, N. Cerf-Bensussan, P. Opolon, N. Yessaad, E. Vivier, B. Ryffel, C. O. Elson, J. Doré, G. Kroemer, P. Lepage, I. G. Boneca, F. Ghiringhelli, L. Zitvogel, The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013).
21. B. Björkholm, C. M. Bok, A. Lundin, J. Rafter, M. L. Hibberd, S. Pettersson, Intestinal microbiota regulate xenobiotic metabolism in the liver. *PLOS One* **4**, e6958 (2009).
22. Y. Eriguchi, S. Takashima, H. Oka, S. Shimoji, K. Nakamura, H. Uryu, S. Shimoda, H. Iwasaki, N. Shimono, T. Ayabe, K. Akashi, T. Teshima, Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of α-defensins. *Blood* **120**, 223–231 (2012).
23. C. M. Paulos, C. Wrzesinski, A. Kaiser, C. S. Hinrichs, M. Chieppa, L. Cassard, D. C. Palmer, A. Boni, P. Muranski, Z. Yu, L. Gattinoni, P. A. Antony, S. A. Rosenberg, N. P. Restifo, Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8$^+$ T cells via TLR4 signaling. *J. Clin. Invest.* **117**, 2197–2204 (2007).
24. J. P. Zackular, N. T. Baxter, K. D. Iverson, W. D. Sadler, J. F. Petrosino, G. Y. Chen, P. D. Schloss, The gut microbiome modulates colon tumorigenesis. *MBiol.* **4**, e00692–e13 (2013).
25. M. T. Liong, Roles of probiotics and prebiotics in colon cancer prevention: Postulated mechanisms and in-vivo evidence. *Int. J. Mol. Sci.* **9**, 854–863 (2008).
26. G. Capurso, M. Marignani, G. Delle Fave, Probiotics and the incidence of colorectal cancer: When evidence is not evident. *Dig. Liver Dis.* **38** (Suppl 2), S277–S282 (2006).
27. L. G. Bermúdez-Humarán, C. Aubry, J. P. Motta, C. Deraison, L. Steidler, N. Vergnolle, J. M. Chatel, P. Langella, Engineering lactococci and lactobacilli for human health. *Curr. Opin. Microbiol.* **16**, 278–283 (2013).
28. J. Pol, N. Bloy, F. Obrist, A. Eggermont, J. Galon, W. Hervé Fridman, I. Cremer, L. Zitvogel, G. Kroemer, L. Galluzzi, Trial Watch: DNA vaccines for cancer therapy. *Oncolmmunology* **3**, e28185 (2014).
29. F. Sandoval, M. Terme, M. Nizard, C. Badoual, M. F. Bureau, L. Freyburger, O. Clement, E. Marcheteau, A. Gey, G. Fraisse, C. Bouguin, N. Merillon, E. Dransart, T. Tran, F. Quintin-Colonna, G. Autret, M. Thiebaud, M. Suleman, S. Riffault, T. C. Wu, O. Launay, C. Danel, J. Taieb, J. Richardson, L. Zitvogel, W. H. Fridman, L. Johannes, E. Tartour, Mucosal imprinting of vaccine-induced CD8$^+$ T cells is crucial to inhibit the growth of mucosal tumors. *Sci. Transl. Med.* **5**, 172ra20 (2013).
30. A. Hamad, K. C. Fragkos, A. Forbes, A systematic review and meta-analysis of probiotics for the management of radiation induced bowel disease. *Clin. Nutr.* **32**, 353–360 (2013).
31. H. S. Taper, M. B. Roberfroid, Possible adjuvant cancer therapy by two prebiotics—Inulin or oligofructose. *In Vivo* **19**, 201–204 (2005).
32. E. Holmes, J. Kinross, G. R. Gibson, R. Burcelin, W. Jia, S. Pettersson, J. K. Nicholson, Therapeutic modulation of microbiota-host metabolic interactions. *Sci. Transl. Med.* **4**, 137rv6 (2012).

Funding: L.Z. and G.K. are supported by the Ligue contre le Cancer (équipe labelisée); Agence Nationale de la Recherche (ANR); Association pour la Recherche sur le Cancer (ARC); Cancéropôle Ile-de-France; AXA Chair for Longevity Research; Institut National du Cancer (INCa); Fondation Bettencourt-Schueller; Fondation de France; Fondation pour la Recherche Médicale (FRM); the European Commission (ArtForce); the European Research Council (ERC); the LabEx Immuno-Oncology; the Site de Recherche Intégrée sur le Cancer (SIRIC) Stratified Oncology Cell DNA Repair and Tumor Immune Elimination (SOCRATE); the SIRIC Cancer Research and Personalized Medicine (CARPEM); and the Paris Alliance of Cancer Research Institutes (PACRI). M.M. is supported by R01 CA154947A, R01 CA173861, U01AI095611, R01AI104848, and U19 AI089987.
Competing interests: The authors declare that they have no competing interests.

10.1126/scitranslmed.3010473

Citation: L. Zitvogel, L. Galluzzi, S. Viaud, M. Vétizou, R. Daillère, M. Merad, G. Kroemer, Cancer and the gut microbiota: An unexpected link. *Sci. Transl. Med.* **7**, 271ps1 (2015).

CANCER

Immunological mechanisms of the antitumor effects of supplemental oxygenation

Stephen M. Hatfield,[1] Jorgen Kjaergaard,[1] Dmitriy Lukashev,[1] Taylor H. Schreiber,[2]* Bryan Belikoff,[1] Robert Abbott,[1] Shalini Sethumadhavan,[1] Phaethon Philbrook,[1] Kami Ko,[1] Ryan Cannici,[1] Molly Thayer,[1] Scott Rodig,[3] Jeffrey L. Kutok,[3†] Edwin K. Jackson,[4] Barry Karger,[5] Eckhard R. Podack,[2] Akio Ohta,[1] Michail V. Sitkovsky[1,6‡]

Antitumor T cells either avoid or are inhibited in hypoxic and extracellular adenosine-rich tumor microenvironments (TMEs) by A2A adenosine receptors. This may limit further advances in cancer immunotherapy. There is a need for readily available and safe treatments that weaken the hypoxia–A2-adenosinergic immunosuppression in the TME. Recently, we reported that respiratory hyperoxia decreases intratumoral hypoxia and concentrations of extracellular adenosine. We show that it also reverses the hypoxia-adenosinergic immunosuppression in the TME. This, in turn, stimulates (i) enhanced intratumoral infiltration and reduced inhibition of endogenously developed or adoptively transfered tumor-reactive CD8 T cells, (ii) increased proinflammatory cytokines and decreased immunosuppressive molecules, such as transforming growth factor–β (TGF-β), (iii) weakened immunosuppression by regulatory T cells, and (iv) improved lung tumor regression and long-term survival in mice. Respiratory hyperoxia also promoted the regression of spontaneous metastasis from orthotopically grown breast tumors. These effects are entirely T cell– and natural killer cell–dependent, thereby justifying the testing of supplemental oxygen as an immunological coadjuvant to combine with existing immunotherapies for cancer.

INTRODUCTION

T lymphocytes and natural killer (NK) cells are inhibited in hypoxic and extracellular adenosine-rich, inflamed (*1*), and cancerous tissues (*2*, *3*) because of immunosuppressive adenosine 3′,5′-monophosphate (cAMP)–mediated signaling, triggered by A2A adenosine receptors (A2ARs). A2ARs interfere with the trafficking and activities of T and NK cells because of the heterologous desensitization of chemokine receptors and reduced proinflammatory cytokines (*2*, *4*). Hypoxia-A2AR–mediated signaling may also recruit and/or further amplify other immunosuppressive mechanisms (*5*, *6*) in the tumor microenvironment (TME), thereby limiting further advances in promising immunotherapies of cancer (*7*–*13*). This may explain the paradoxical coexistence of tumors and antitumor T cells in cancer patients and mice (*6*, *14*). This view is supported by findings of enhanced T cell– and NK cell–mediated tumor rejection in mice that are genetically deficient in A2AR (*2*,*15*) or adenosine-generating CD39/CD73 (*13*, *16*–*19*) or in the presence of A2AR antagonists (*2*, *19*, *20*). In addition, the overexpression of extracellular adenosine-generating CD73 on human breast tumors and A2 adenosine receptors on antitumor immune cells was implicated in the protection of tumors from chemotherapy and immunotherapy (*21*).

Thus, there is strong justification and motivation to develop safe treatments that weaken the hypoxia-driven and CD39/CD73-mediated accumulation of extracellular adenosine and immunosuppressive signaling through A2AR on T and NK cells in the TME by targeting CD39 or CD73 ectoenzymes (*13*, *16*–*19*) or by antagonizing the A2AR (*2*, *3*, *6*, *13*). We hypothesized that the reduction of tumor hypoxia using clinical supplemental oxygen protocols (*22*–*24*) may inhibit the hypoxia-driven accumulation of extracellular adenosine in the TME (*25*) and weaken the A2AR-mediated immunosuppression. This, in turn, may further improve cancer immunotherapy approaches and enable tumor regression by unleashing antitumor T and NK cells.

To test this hypothesis, tumor-bearing mice were placed in chambers with well-controlled gas composition (60% oxygen) to mimic protocols of supplemental oxygen delivery to humans (*22*–*24*). This immunological mechanism-based motivation to use respiratory hyperoxia (60% oxygen) as an anti-adenosinergic treatment is conceptually different from the classic approach to generate reactive oxygen species (ROS) in radiotherapy and photodynamic therapy of cancer by breathing 95% O_2/5% CO_2 (carbogen) (*26*–*29*).

RESULTS

Respiratory hyperoxia has antitumor effects

In studies of the weakly immunogenic MCA205 fibrosarcoma pulmonary tumor model with a predictable time course and intensity of T cell response (*30*, *31*), mice breathing 60% oxygen demonstrated improved regression of lung tumors (Fig. 1A). Tumor regression was observed in mice with established lung tumors (11 days) treated with respiratory hyperoxia, long after tumor inoculation (day 11, identified in Fig. 1A as "60% O_2*"). An even stronger regression was observed when mice were treated with respiratory hyperoxia starting immediately after tumor inoculation until assay completion on day 21 (identified as "60%

[1]New England Inflammation and Tissue Protection Institute, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA. [2]Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL 33136, USA. [3]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, 20 Shattuck Street, Boston, MA 02115, USA. [4]Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219, USA. [5]Barnett Institute of Chemical and Biological Analysis, Northeastern University, Boston, MA 02115, USA. [6]Cancer Vaccine Center, Dana-Farber Cancer Institute, Harvard Institutes of Medicine, 44 Binney Street, Boston, MA 02115, USA.
*Present address: Heat Biologics Inc., 801 Capitola Drive, Durham, NC 27713, USA.
†Present address: Infinity Pharmaceuticals Inc., 780 Memorial Drive, Cambridge, MA 02139, USA.
‡Corresponding author. E-mail: m.sitkovsky@neu.edu

O_2"). Hyperoxia-induced tumor regression was also observed in the poorly immunogenic B16 melanoma pulmonary tumor model (Fig. 1B). Using a less aggressive model of induced metastasis, respiratory hyperoxia (60% O_2) commencing after tumor inoculation resulted in the complete regression of lung tumors and survival of 40% of mice compared to mice breathing ambient 21% O_2 (Fig. 1C). The data in Fig. 1D also demonstrate reduced lung tumor nodules in the spontaneously metastasizing 4T1 triple-negative breast cancer (TNBC) model when mice with 7-day established orthotopic tumors were treated with respiratory hyperoxia. The 4T1 tumor cells express high levels of the adenosine-generating ectoenzyme CD73 to mimic drug-resistant TNBC (*21*).

These data provide support for the clinical testing of anti–A2-adenosinergic interventions, including systemic oxygenation (*25*), as treatments for chemotherapy-resistant TNBC. Indeed, the overexpression of CD73 by TNBC and subsequent adenosine-A2AR/A2BR–mediated immunosuppression in the TME was shown to contribute to resistance to chemotherapy in the analysis of more than 6000 triple-negative and chemotherapy-resistant breast cancers (*21*).

The antitumor effects of respiratory hyperoxia require the activities of endogenous T and NK cells

We tested whether the observed antitumor effects of respiratory hyperoxia were mediated by the increased activities of endogenously developed tumor-reactive T and NK cells or, alternatively, represent the result of direct cytotoxicity or oxidative stress–mediated tumor damage caused by ROS generated by hyperoxia (*26*–*29*) or formed independently of hyperoxia during purine metabolism.

To discriminate between these mechanisms, we tested whether the hyperoxia-induced tumor regression would still be observed in mice genetically deficient in T and NK cells (*24*) [common gamma (γc)/*Rag-2*$^{-/-}$ mice] or in tumor-bearing wild-type mice treated with ROS scavengers. It was expected that if the antitumor effects were the result of ROS generated by 60% oxygen, then respiratory hyperoxia would be capable of inducing tumor regression even in the absence of T and/or NK cells.

Figure 2A and fig. S1 demonstrate that the improved tumor regression seen in wild-type mice breathing 60% oxygen was lost in γc/*Rag-2*$^{-/-}$ mice breathing 60% oxygen. This established the necessity of tumor-reactive T and NK cells for the hyperoxia-enhanced antitumor response. These data also serve as genetic controls indicating that hyperoxia has no effect on tumor seeding or colonization, because breathing 60% oxygen immediately after tumor inoculation did not reduce the number or size of lung tumors in γc/*Rag-2*$^{-/-}$ mice. Similarly, the tumor-regressing effects of hyperoxia were observed in wild-type mice with established tumors even when hyperoxic exposure began after the 11th day of tumor growth, after seeding and colonization (Fig. 1A). Independent confirmation of the lack of effects of 60% oxygen–generated ROS was provided by testing the effects of a ROS scavenger [*N*-acetylcysteine (NAC)] on lung tumor growth in mice treated with respiratory hyperoxia (*26*–*29*). Figure S2

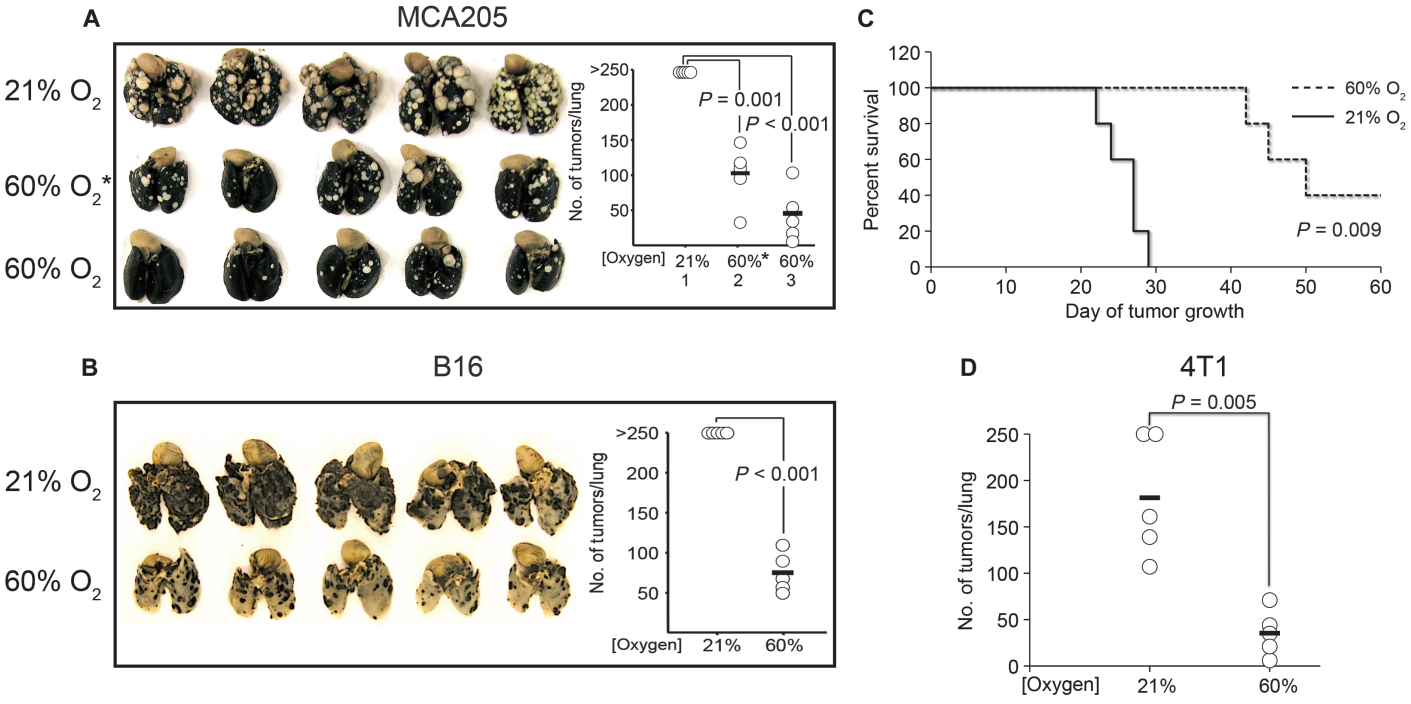


Fig. 1. Respiratory hyperoxia promotes tumor regression and survival and decreases metastasis. (**A**) Respiratory hyperoxia promotes tumor regression in mice with 11-day established MCA205 pulmonary tumors. Mice were placed in chambers with 60% oxygen after 11 days of tumor growth (identified as 60% O_2*; $P = 0.001$), and lungs were harvested at day 21. Stronger regression was observed when mice were placed in 60% oxygen units immediately after tumor inoculation (identified as 60% O_2; $P = 0.0003$) ($n = 5$ mice per group, averages represented as horizontal bars). (**B**) Hyperoxia-enhanced tumor regression in mice with B16 melanoma pulmonary tumors ($n = 5$ mice per group, averages represented as horizontal bars; $P = 0.0001$). (**C**) Respiratory hyperoxia leads to long-term survival in 40% of MCA205 tumor–bearing mice ($n = 5$ mice per group; $P = 0.009$). (**D**) Respiratory hyperoxia strongly decreases spontaneous lung metastasis of orthotopically grown 4T1 breast tumors ($n = 5$ mice per group; $P = 0.005$). Balb/c mice were injected in the third mammary fat pad with 4T1 tumor cells. After tumors became palpable at day 7, mice were placed in either 21 or 60% oxygen until assay completion on day 28.

demonstrates that daily treatment with NAC at doses that are shown to reduce ROS in positive control assays (fig. S2A) does not significantly inhibit the tumor-regressing effects of respiratory hyperoxia (fig. S2B).

To determine the relative contribution of different immune cell subsets in the hyperoxia-induced tumor regression, wild-type mice were depleted of T and NK cells using monoclonal antibodies (mAbs). Figure 2B shows that the improved regression of pulmonary tumors by 60% oxygen is mediated to a large extent by endogenous T cells, because mice depleted of CD4 and CD8 cells demonstrated severely impaired tumor regression after respiratory hyperoxia.

The depletion of NK cells alone virtually eliminated the antitumor effects of respiratory hyperoxia, although NK cell–depleted mice still retained CD4 and CD8 T cells. Although it has been well established that NK cells are important in antitumor immunity (*32*, *33*), our data suggest that the full antitumor potential of NK cells may not be realized because of hypoxia–A2-adenosinergic inhibition in the TME. These in vivo observations also extend previous in vitro demonstrations (*4*) of the high susceptibility of NK cells to A2AR-mediated inhibition. This is further supported by the data in fig. S3, demonstrating hypoxia and A2AR-mediated inhibition of NKG2D expression, NK cell activation, and cytokine secretion.

Thus, respiratory hyperoxia may enhance the antitumor activities not only of T cells but also of NK cells. These data extend previous observations of the critical importance of NK cells in enabling T cell–mediated tumor regression under normal oxygen conditions (*32*, *33*).

Respiratory hyperoxia acts upstream of the hypoxia-adenosinergic pathway

Because we recently established that breathing 60% oxygen was capable of reducing hypoxia and adenosine in the TME (*25*), we hypothesized that respiratory hyperoxia might block the upstream stage of the hypoxia-adenosine-A2AR–mediated immunosuppressive pathway in the TME (*6*). If so, then hyperoxia would not be able to further improve tumor regression in $A2AR^{-/-}$ mice as compared to wild-type mice (*2*). In agreement with this hypothesis, Fig. 2C (right side) demonstrates that respiratory hyperoxia did not further improve tumor regression in $A2AR^{-/-}$, suggesting that the reversal of hypoxia was acting upstream of A2AR signaling. This conclusion was confirmed in adoptive transfer experiments, where respiratory hyperoxia enhanced the therapeutic efficacy of wild-type T cells, but not $A2AR^{-/-}$ T cells (fig. S4). Data from Fig. 2C (identified as "21% WT" versus "21% $A2AR^{-/-}$") also extend previous observations of the enhanced rejection of intradermal tumors in $A2AR^{-/-}$ mice (*2*) to the pulmonary foci tumor model. Together, data in Figs. 1 and 2 provide genetic evidence that hyperoxia prevents the inhibition of antitumor T and NK cells by acting upstream of the hypoxia-$[adenosine]^{High}$-A2AR-$[cAMP]^{High}$ pathway.

In support of the hypothesis that the reversal of hypoxia in the TME prevents the inhibition of antitumor immunity, analysis of tumors in different anatomical locations shows markedly fewer CD8 and CD4 T cells in hypoxic areas of tumors compared to normoxic neighboring regions of the same tumor (Fig. 3A and fig. S5). This demonstration that T cells seem to avoid hypoxia provides an explanation of the limited tumor infiltration by T cells and the less than optimal clinical outcomes of the immunotherapies in cancer (*34*).

Using a molecular in vivo hypoxia marker (*25*), Fig. 3 (B and C) demonstrate that respiratory hyperoxia reduced the exposure of lymphocytes to hypoxia in the TME as well as in lymphoid organs. Both CD8 and CD4 T cells from the lungs and spleen of tumor-bearing mice breathing 60% oxygen had less hypoxic staining. These observations extend and support our previous data demonstrating that respiratory hyperoxia decreased intratumoral hypoxia and the concentrations of extracellular adenosine in the TME (*25*).

Respiratory hyperoxia converts an immunosuppressive TME into an immunopermissive TME

Additional analysis of the TME demonstrated that the conversion to an immunopermissive TME by respiratory hyperoxia resulted in the highly desirable (*34*) enhancement of tumor infiltration by antitumor CD8 T cells (Fig. 4A). This was confirmed and extended in a flow cytometric time course assay showing the hyperoxia-enhanced accumulation of highly activated CD8 T cells in the pulmonary TME (Fig. 4B). Mice with established pulmonary tumors treated with respiratory hyperoxia demonstrated an increase in the number of $CD8^+$, $CD69^+$, and $CD44^+$ cells in the TME. Because T cells demonstrate aversion to hypoxia (Fig. 3A), the reduction in the exposure of T cells to hypoxia in the TME after hyperoxic breathing may explain their increased presence

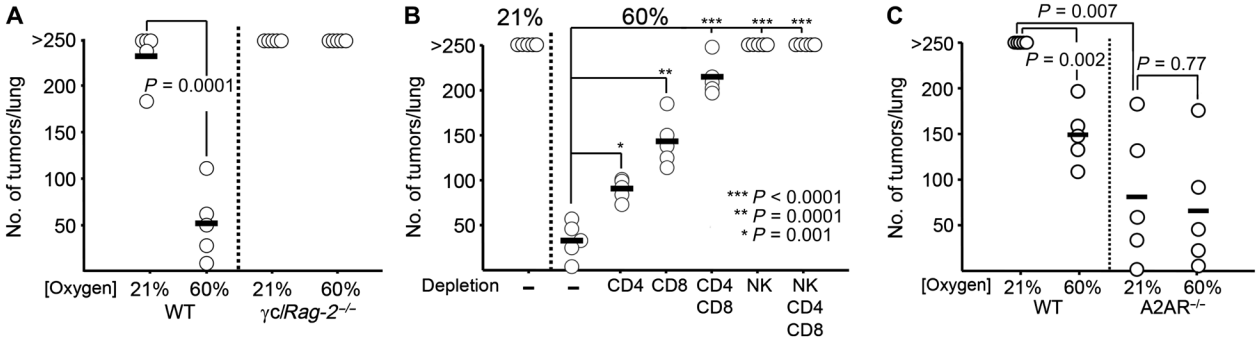


Fig. 2. Antitumor effects of respiratory hyperoxia require endogenous T and NK cells. (**A**) Tumor-regressing effects of hyperoxia are lost in γc/*Rag-2*$^{-/-}$ mice deficient in T and NK cells. MCA205 tumor–bearing wild-type (WT) or γc/*Rag-2*$^{-/-}$ mice were placed in 21 or 60% oxygen after tumor inoculation, and lung tumors were assessed after 21 days ($n = 5$ mice per group, averages represented as horizontal bars; $P = 0.0001$). (**B**) Hyperoxia-induced regression of MCA205 pulmonary tumors is mediated by CD4, CD8, and NK cells. Depletion of different T cell subsets or NK cells using mAbs 2 days before tumor inoculation impaired or completely abrogated the antitumor effects of 60% oxygen ($n = 5$ mice per group, averages represented as horizontal bars; $^*P = 0.001$, $^{**}P = 0.0001$, $^{***}P < 0.0001$). (**C**) Respiratory hyperoxia improves tumor regression in MCA205 tumor–bearing WT mice but does not significantly improve the therapeutic benefit of genetic elimination of A2AR ($n = 5$ mice per group, averages represented as horizontal bars; $P = 0.002$ and $P = 0.77$ for WT and $A2AR^{-/-}$, respectively).

in the TME. In these assays, the recruitment of CD4 T cells was not affected, suggesting that respiratory hyperoxia may differentially affect CD8 versus CD4 T cells. This could be due to changes in the cytokine/chemokine profile in the TME after respiratory hyperoxia or differences in the expression of chemokine receptors.

In Fig. 4 (C and D), we used custom-made reverse transcription polymerase chain reaction (RT-PCR) arrays to scan the pulmonary TME for hyperoxia-induced changes among 94 proinflammatory and tolerogenic mediators, including 4 chemokine receptors, 20 chemokine ligands, and 27 different cytokines and chemokines (*35*). The hyperoxia-associated increase in the levels of proinflammatory cytokines [IL-2 (interleukin-2) and IL-12] and chemokines [CXCL9 (CXC motif ligand 9), CXCL10, and CXCL11] (Fig. 4C) was accompanied by the simultaneous decrease in the immunosuppressive cytokine transforming growth factor–β (TGF-β) (Fig. 4D).

Respiratory hyperoxia may weaken immunosuppression by regulatory T cells in the TME

Experiments in Fig. 5A addressed a well-appreciated problem in clinical immunotherapy protocols, which is the presence of suppressive regulatory T cells (T_{regs}) in the TME that inhibit the antitumor immune response (*36*). We hypothesized that respiratory hyperoxia may decrease immunosuppression by T_{regs} in the TME because of the proposed role of hypoxia and cAMP response element (HRE/CRE)–mediated transcription in the development and function of T_{regs} (*5*). To this end, we analyzed the effect of respiratory hyperoxia on the time course of $CD4^+$, $CD25^+$, $Foxp3^+$ T_{reg} tumor infiltration and the expression of negative regulators of the immune response.

Data from Fig. 5 suggest that hyperoxia weakens T_{reg}-mediated suppression of the immune response in the pulmonary TME by four distinct mechanisms. Respiratory hyperoxia resulted in (i) a decrease in the percentage of T_{regs} in the pulmonary TME (Fig. 5A, left), (ii) reduced levels of the transcription factor Foxp3 in T_{regs} (Fig. 5A, right), (iii) reduced expression of the adenosine-generating enzymes CD39/CD73 on T_{regs} (Fig. 5, B and C), and (iv) a decrease in the expression of CTLA-4 (cytotoxic T lymphocyte–associated protein 4) on T_{regs} (Fig. 6A), which has been shown to be critical for T_{reg}-mediated suppression (*37*).

In accordance with data from Fig. 3 on CD4 and CD8 T cells in the TME, T_{regs} from tumor-bearing mice breathing 60% oxygen demonstrated less exposure to hypoxia (Fig. 6B). Because respiratory hyperoxia reduced CTLA-4 on T_{regs} (Fig. 6A) and CTLA-4 is important for the functions of T_{regs} (*37*), the relationship between hypoxia and CTLA-4 expression on T_{regs} was further evaluated. Figure 6C and fig. S6 demonstrate that CTLA-4^{High} T_{regs} from the lung TME and spleen of tumor-bearing mice were exposed to lower oxygen tension when compared to CTLA-4^{Low} T_{regs}. Although both CTLA-4^{High} and CTLA-4^{Low} T_{regs} were present in mice breathing 21% and 60% oxygen, the hypoxia staining from the CTLA-4^{High} T_{reg} population was lower in hyperoxia-treated mice. This,

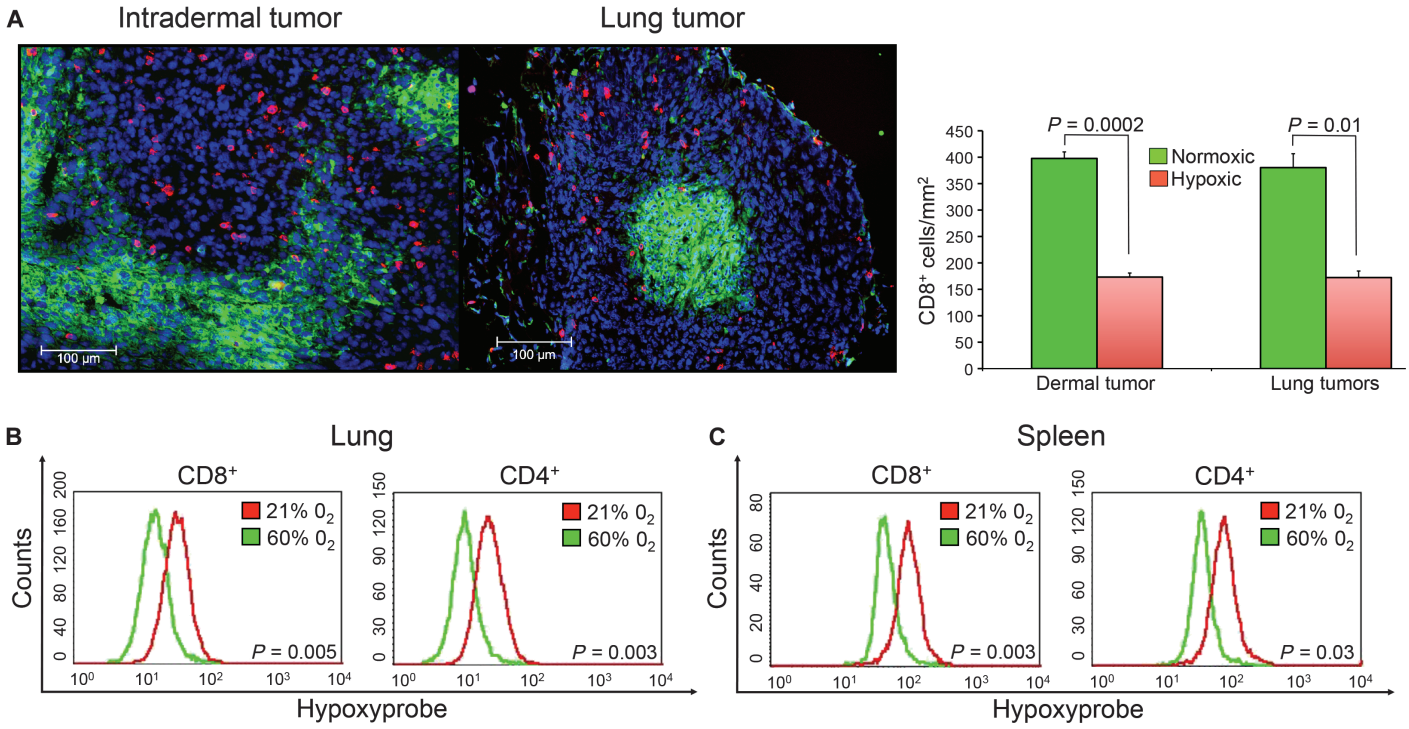


Fig. 3. Antitumor T cells avoid hypoxic areas of the TME. (**A**) Immunohistochemical demonstration of CD8 T cells (red) preferentially localized outside of hypoxic areas (green) of intradermal (left panel) and lung (right panel) TME. Tissue sections from 14-day established lung or intradermal MCA205 tumors were analyzed. Statistical comparison between hypoxic and normoxic locations of CD8 T cells seen in the representative images on the left (scale bar, 100 μm) is shown in the histogram on the right (dermal: $n = 3$ mice, $P = 0.0002$; lung: $n = 3$ mice, $P = 0.01$). (**B**) Respiratory hyperoxia decreases hypoxic exposure of CD4 and CD8 T cells in the lung TME and spleen of tumor-bearing mice. Lymphocytes were isolated from MCA205 tumor–bearing lungs or spleen of mice breathing 21 or 60% oxygen, and the mean fluorescence intensity (MFI) of Hypoxyprobe-labeled T cells was analyzed by flow cytometry (lung: $n = 4$ mice per group; CD8 $P = 0.005$, CD4 $P = 0.003$; spleen: $n = 3$ mice per group; CD8 $P = 0.003$, CD4 $P = 0.03$).

in turn, may inhibit HRE- and CRE-mediated immunosuppressive transcription of suppressive mediators, such as TGF-β (Fig. 4D) (*5*).

To extend the studies of endogenously developed tumor-reactive T cells and to gain additional mechanistic insights into hyperoxia-mediated enhancement of tumor regression, we studied adoptively transferred tumor-reactive T cells in mice breathing 60% oxygen. Mice with 11-day established pulmonary tumors were infused intravenously with in vitro culture-activated antigen-specific T cells (fig. S7) derived from tumor-draining lymph nodes (TDLNs). Twenty-four hours before transfer, mice were treated with cyclophosphamide to mimic clinical protocols of adoptive cell transfer (*30*, *31*). As shown in Fig. 7A, commencing respiratory hyperoxia on the same day as adoptive T cell immunotherapy in mice with 11-day established tumors enhanced tumor regression when compared to mice treated with T cells alone (Fig. 7A; 60%*). An even stronger therapeutic effect, shown by the complete regression of lung tumors by adoptively transferred tumor-reactive T cells, was achieved if mice were breathing 60% oxygen from the time of tumor inoculation until the assay completion on day 21 (Fig. 7A; 60%). In control assays, adoptively transferred tumor-reactive $A2AR^{-/-}$ T cells demonstrated no improved efficacy when combined with respiratory hyperoxia (fig. S4).

Because limited tumor infiltration of antitumor T cells has been shown to diminish the effects of immunotherapy, we examined the trafficking of adoptively transferred tumor-reactive T cells in the TME. Figure 7B shows the facilitation of intratumoral infiltration and the increased number of carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled adoptively transferred T cells in pulmonary tumors of mice treated with respiratory hyperoxia. These results are complementary to and extend the observation of increased intratumoral infiltration of endogenously developed CD8 T cells in mice breathing 60% oxygen (Fig. 4, A and B). Respiratory hyperoxia also increased the production of interferon-γ (IFN-γ) by adoptively transferred ($Thy1.1^+$) and endogenously developed ($Thy1.2^+$) tumor-reactive T cells from the pulmonary TME (fig. S8).

Because data from Fig. 2 (A and B) pointed to the importance of NK cells in the hyperoxia-induced tumor regression, we also examined the effect of respiratory hyperoxia on adoptively transferred activated NK cells. Figure S3D demonstrates that respiratory hyperoxia enhanced the tumoricidal activities of transferred NK cells against established B16 pulmonary tumors. Confirming and extending data from Fig. 1B, respiratory hyperoxia improved tumor regression of established B16 tumors even in the absence of NK cell transfer. However, combination with adoptive transfer of NK cells resulted in the strongest tumor regression. The hyperoxia-mediated enhancement of NK cell activity occurred without adjunctive IL-2 therapy, which is often critical for effective NK cell therapy (*38*).

Additional studies also determined whether the antitumor effects of supplemental oxygen could be accomplished by less than 60% oxygen. Figure 7C demonstrates in dose-response studies that breathing as low as 40% oxygen was also capable of promoting tumor regression. By alternating the breathing of 60% oxygen with 40 or 21% oxygen every 12 hours, we established that breathing 60% oxygen 24 hours/day was

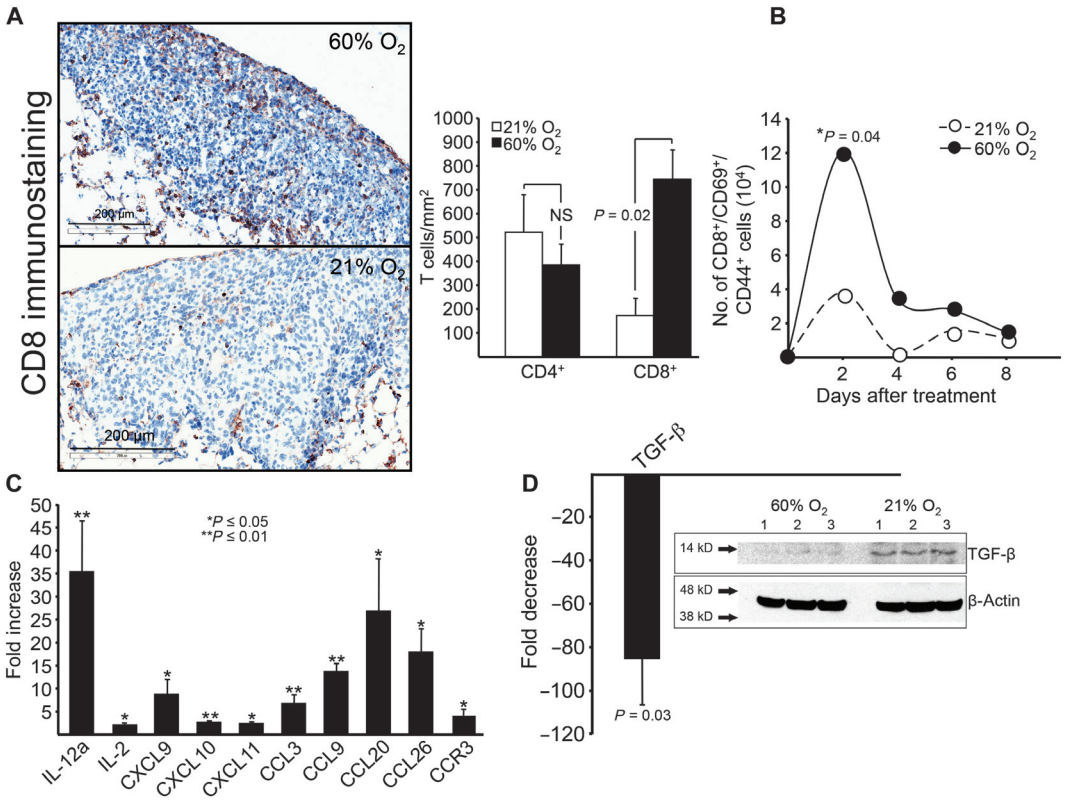


Fig. 4. Respiratory hyperoxia results in an immunopermissive TME. (**A**) Immunohistochemical demonstration of the enhanced infiltration of endogenous CD8 T cells into established MCA205 pulmonary tumors due to hyperoxic breathing (means ± SEM, $P = 0.015$; $n = 3$ mice per group; scale bar, 200 μm). (**B**) Hyperoxic breathing promotes the accumulation of highly activated endogenous CD8 T cells as shown by flow cytometric analysis of the pulmonary TME. Mice with 11-day established MCA205 pulmonary tumors were treated with respiratory hyperoxia for 4 days, and the number of $CD8^+$, $CD69^+$, and $CD44^+$ cells was analyzed by flow cytometry (means ± SEM, $^*P = 0.04$; $n = 3$ mice per group). (**C**) Respiratory hyperoxia increases the levels of immunostimulating cytokines and chemokines as detected using custom-made RT-PCR arrays to screen for changes in 94 different chemokines and cytokines. Mice with 11-day established MCA205 pulmonary tumors were treated with respiratory hyperoxia for 72 hours (means ± SEM, exact *P* values listed in table S1; $n = 3$ mice per group). (**D**) Respiratory hyperoxia decreases the levels of TGF-β in the lung TME (means ± SEM, $P = 0.03$; $n = 3$ mice per group). Inset: Immunoblot for TGF-β in lung tumors from mice breathing 21 and 60% oxygen. Mice with 11-day established MCA205 pulmonary tumors were treated with respiratory hyperoxia for 72 hours.

necessary to achieve the strongest antitumor effects (Fig. 7D). Because CTLA-4/PD-1 (programmed cell death protein 1) blockade represents one of the most important recent advances in cancer immunotherapy (*8*–*12*), we also tested whether respiratory hyperoxia would be capable of enhancing the therapeutic efficacy of CTLA-4/PD-1 blockade against pulmonary tumors. Figure 7E demonstrates that the tumor regression induced by dual blockade of CTLA-4/PD-1 was enhanced by respiratory hyperoxia.

DISCUSSION

This study demonstrates that the weakening of upstream tumor hypoxia by supplemental oxygenation decreases the intensity of the downstream A2AR-mediated immunosuppression in the TME. This, in turn, releases the restraints of the otherwise inhibited antitumor activities of T and NK cells and enables tumor regression and survival.

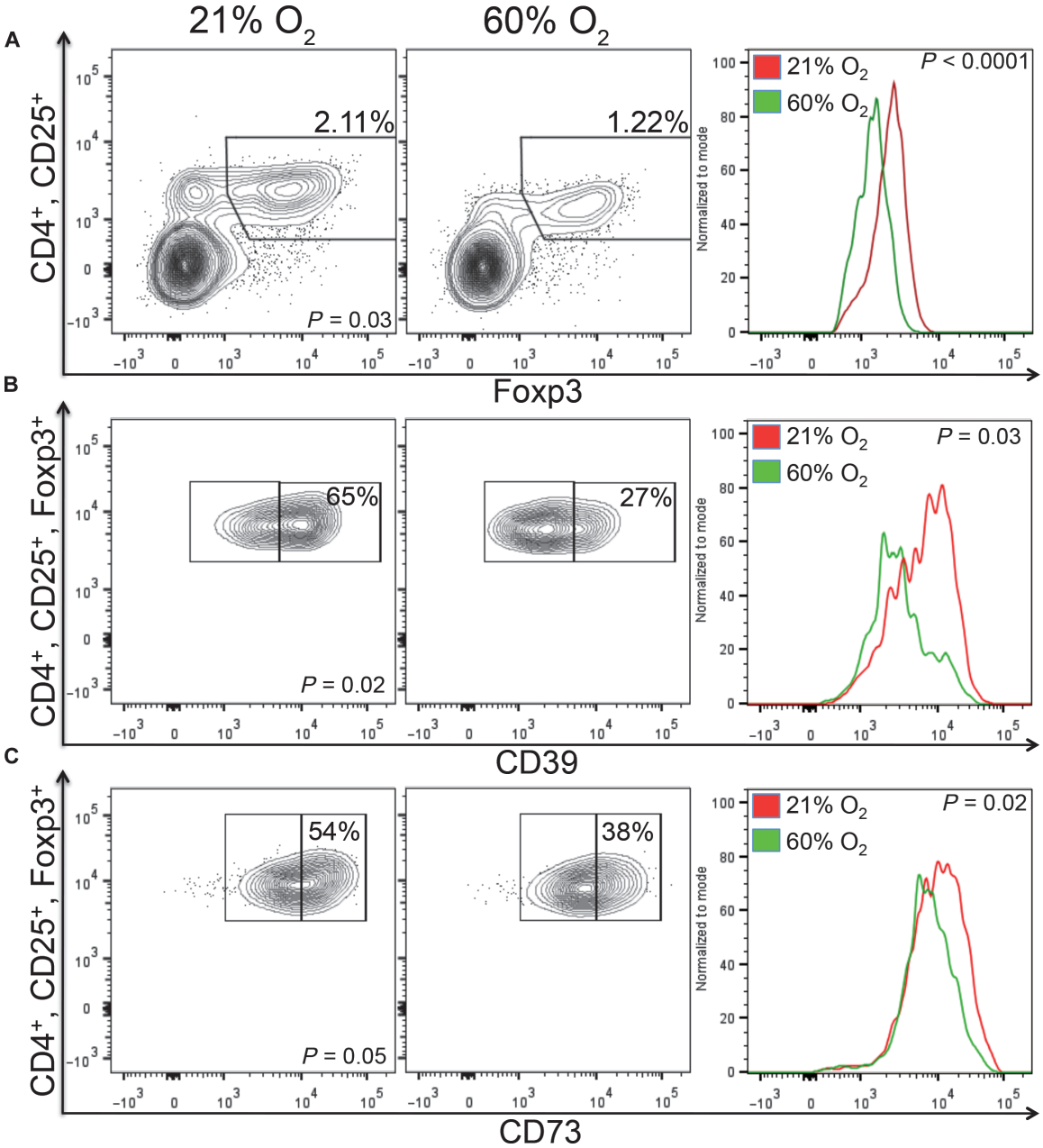


Fig. 5. Respiratory hyperoxia weakens immunosuppression by T_{regs} in the lung TME. (**A**) Left: Respiratory hyperoxia decreases the percentage of $CD4^+/CD25^+/Foxp3^+$ T_{regs} in the lung TME ($P = 0.03$; $n = 5$ mice per group). Mice bearing 11-day established MCA205 pulmonary tumors were placed in either 21 or 60% oxygen for 72 hours. Right: The expression of Foxp3 was also reduced by respiratory hyperoxia. The average MFI was 2227 and 1572 in mice breathing 21% versus 60% oxygen, respectively ($P = 5.82 \times 10^{-5}$; $n = 5$ mice per group). (**B** and **C**) Left: Respiratory hyperoxia reduces the expression of CD39 (B) and CD73 (C) on T_{regs} in the TME ($P = 0.02$ and $P = 0.05$; $n = 5$ mice per group). Right: The following are the average MFIs from 21 and 60% oxygen, respectively: CD39 (6329, 4226; $P = 0.03$) and CD73 (17054, 15761; $P = 0.02$).

These previously unappreciated and potentially medically valuable immunoenhancing antitumor effects of 60% oxygen were observed because of the assumption that oxygenation must be combined with the parallel activities of tumor-reactive T and NK cells. Indeed, these data demonstrate that the tumor-regressing effects of respiratory hyperoxia are dependent on the presence of T and NK cells. In accordance with earlier studies focused on the mechanisms of cytotoxicity, these data suggest that the improved tumor rejection could be accounted for by exocytosis of perforin-containing granules and by FAS-mediated cytotoxicity of antitumor T and NK cells unleashed by the weakening of hypoxia-adenosinergic immunosuppression (*39*). We recently demonstrated that respiratory hyperoxia induced the up-regulation of major histocompatibility complex class I on tumor cells, enhancing T cell–mediated cytotoxicity (*25*). This resulted in the increased recognition and destruction of tumor cells in vitro (*25*). Additionally, respiratory hyperoxia increased the production of proinflammatory cytokines and chemokines, including IFN-γ. This may contribute to starvation-induced apoptotic tumor cell death, potentially mediated by the increased levels of IFN-γ (*2*).

The use of respiratory hyperoxia offers a feasible direction in attempts to improve the immunotherapy of cancer by inhibiting not only the tumor-protecting hypoxia-CD39/CD73–mediated accumulation of immunosuppressive extracellular adenosine but also the hypoxia-driven formation of intracellular adenosine (*40*–*42*). This is another important potential source of extracellular adenosine (*40*–*42*). Hypoxia may increase the formation of intracellular adenosine by (i) decreasing intracellular levels of adenosine triphosphate, (ii) increasing intracellular AMP, (iii) inhibiting adenosine kinase, and (iv) increasing the expression of 5′-nucleotidase (*40*–*42*). Indeed, hypoxia/HIF-1α (hypoxia-inducible factor–1α)–driven inhibition of adenosine kinase leads to the accumulation of intracellular adenosine (*41*). This, in turn, may elevate levels of extracellular adenosine independent of CD39/CD73, further contributing to immune suppression (*16*–*19*, *21*, *42*).

It remains a possibility that suppression by tumor-associated macrophages (TAMs) or myeloid-derived suppressor cells (MDSCs) (*43*–*45*) might also be altered by the reversal of hypoxia. It has been well established that these cell types play an important role in tumor growth, metastasis, and suppression of the antitumor immune response. Moreover, it has recently been shown that hypoxia and HIF-1α drive the recruitment of MDSCs and TAMs to the TME, as well as M2-like polarization and activity (*43*–*45*).

Among the limitations of this study are the yet to be fully understood mechanisms of oxygenation-mediated de-inhibition of NK cells and

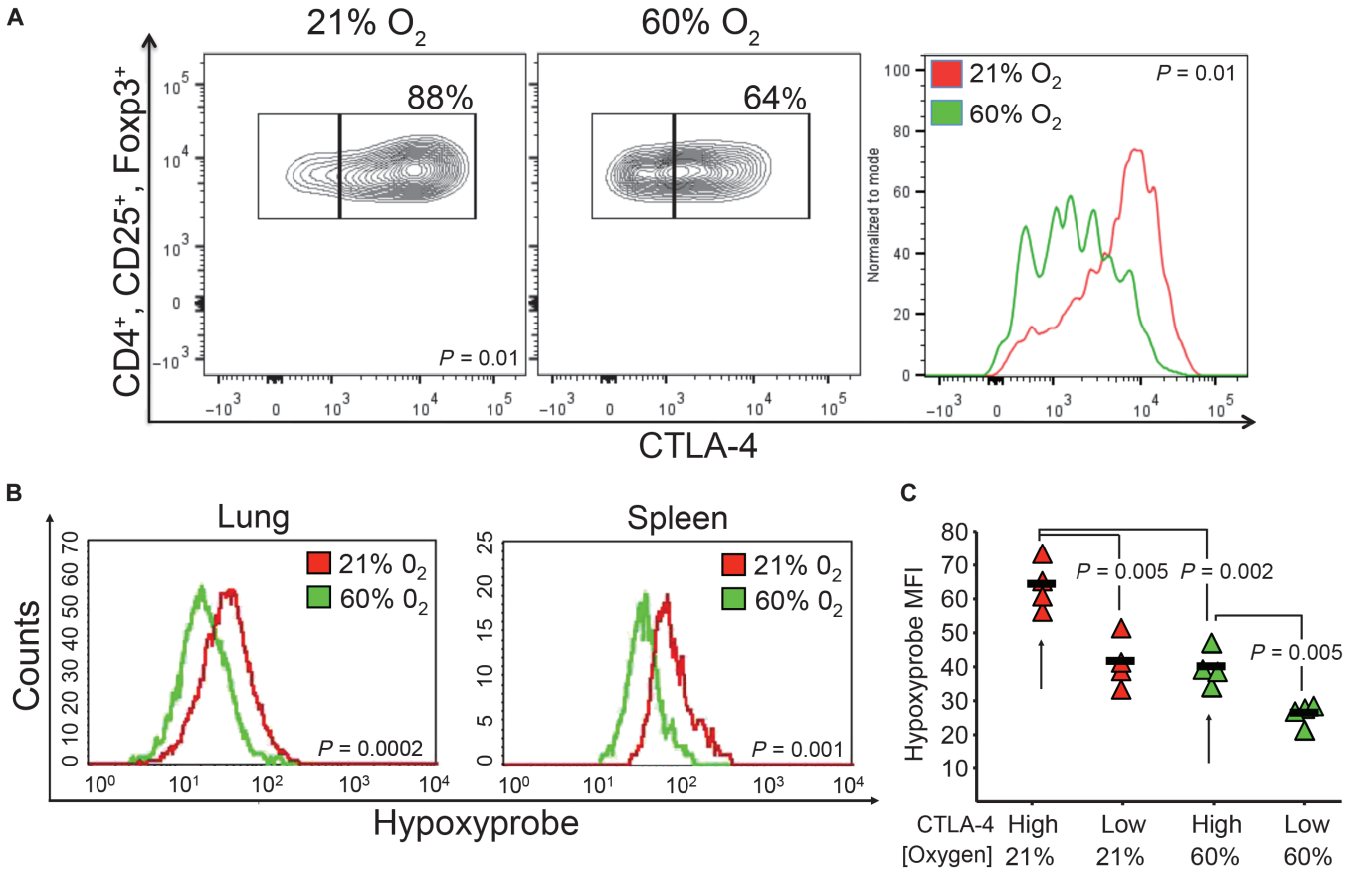


Fig. 6. Respiratory hyperoxia decreases exposure of T_{regs} to hypoxia and reduces expression of CTLA-4. (**A**) Respiratory hyperoxia reduces the expression of CTLA-4 by T_{regs}. The average MFI of CTLA-4 on T_{regs} was 4786 in mice breathing 21% O_2 and 2684 in mice breathing 60% O_2 ($P = 0.01$; $n = 5$ mice per group). (**B**) Respiratory hyperoxia reduces the exposure of T_{regs} to hypoxia in both the lung and the spleen of MCA205 tumor–bearing mice (lung: $P = 0.0002$, $n = 4$ mice per group; spleen: $P = 0.001$, $n = 3$ mice per group). (**C**) CTLA-4^{High} T_{regs} in the lung TME were also Hypoxyprobe^{High}, reflecting in vivo exposure to deeper levels of hypoxia. Respiratory hyperoxia decreased the numbers of CTLA-4^{High} T_{regs} compared to mice breathing 21% oxygen ($P = 0.002$; $n = 4$ mice per group).

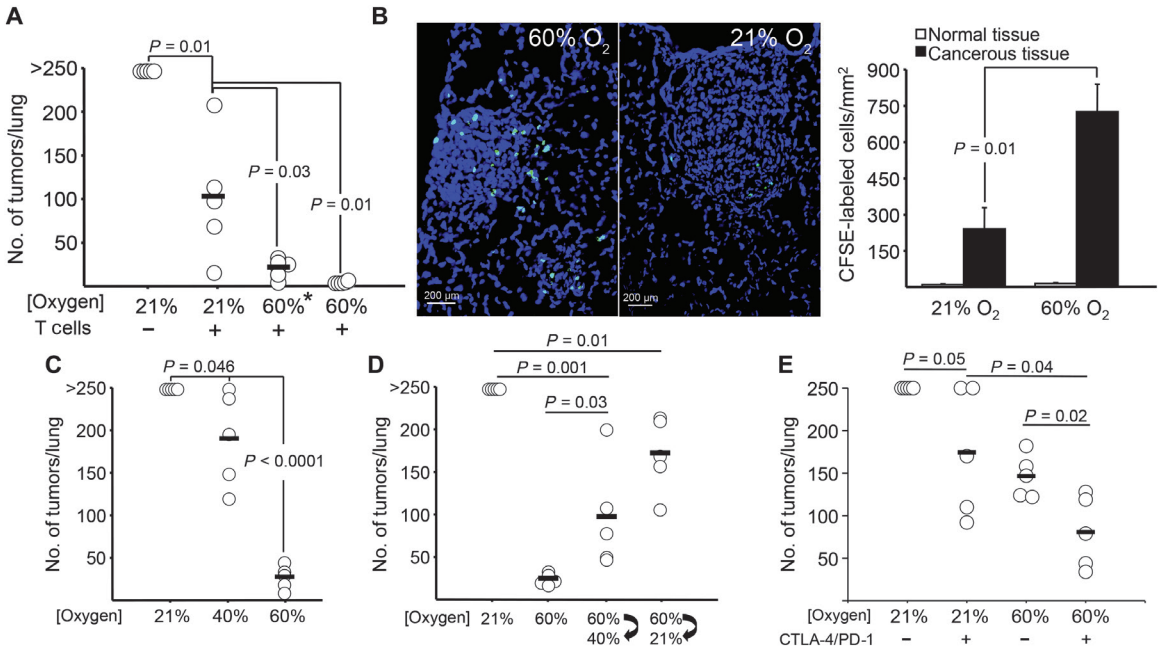


Fig. 7. Respiratory hyperoxia improves tumor regression in preclinical models of immunotherapies. (**A**) Adoptive immunotherapy in combination with respiratory hyperoxia enabled the complete regression of 11-day established MCA205 pulmonary tumors. Mice identified as 60%* were placed in the 60% oxygen units the same day as adoptive T cell immunotherapy, whereas mice identified as 60% were placed in oxygen units for the duration of the assay (21 days) [$n = 5$ mice per group, averages represented as horizontal bars; $P = 0.03$ (60%*) and $P = 0.01$ (60%)]. (**B**) Hyperoxia facilitates the infiltration of adoptively transferred T cells into 11-day established pulmonary tumors. Left: Fluorescent micrographs (scale bar, 200 μm) of CFSE-labeled adoptively transferred T cells (green) in mice breathing 60 or 21% oxygen 48 hours after adoptive transfer. Right: Enumeration of tumor-infiltrating transferred T cells. The average infiltration from ~100 tumors was 249 cells/mm^2 in control mice and 723 cells/mm^2 in mice breathing 60% oxygen ($n = 3$ mice per group; means ± SEM, $P = 0.01$). (**C**) Breathing as low as 40% oxygen results in pulmonary tumor regression [$n = 5$ mice per group, averages represented as horizontal bars; $P = 0.046$ (40% O_2) and $P = 3 \times 10^{-6}$ (60% O_2)]. (**D**) Alternating between breathing 60 and 40% oxygen or 60 and 21% oxygen every 12 hours enables tumor regression compared to mice continuously breathing 21% oxygen ($n = 5$ mice per group, averages represented as horizontal bars; $P = 0.001$ and $P = 0.01$, respectively). Breathing 60% oxygen continuously (24 hours/day) causes the strongest antitumor activity. (**E**) Respiratory hyperoxia improves the outcome of dual CTLA-4/PD-1 blockade in preclinical studies of lung tumor rejection. Mice were inoculated with MCA205 tumor cells and given mAbs for CTLA-4/PD-1 intraperitoneally on days 3, 6, and 9 (500 μg). Mice were treated with respiratory hyperoxia from days 3 to 21 or maintained at 21% O_2 until assay completion (day 21) ($n = 5$ mice per group, averages represented as horizontal bars; $P = 0.04$).

their potential role in orchestrating antitumor T cell responses. In addition, it is not yet clear whether the weakening of TME hypoxia in vivo affects the repertoire of activating versus inhibitory NK ligands.

An additional limitation exists in the requirement of 24-hour/day respiratory hyperoxia to accomplish the maximal outcome in these preclinical studies. However, the continuing advances in the design and use of high-oxygen masks may increase patient compliance with protocols of respiratory hyperoxia. A promising solution would be to decrease the required treatment time of respiratory hyperoxia by combining it with synthetic A2AR antagonists to further prevent immunosuppressive A2AR signaling by tumor-produced adenosine.

Although it is established that high levels of supplemental oxygen (>95% O_2) can cause oxygen toxicity as well as nonspecific inflammatory responses, the use of 60% oxygen is not associated with high-oxygen toxicity and is considered to be safe in long-term treatments (*22*). However, the delivery of 60% oxygen should be considered with an understanding that although long-term treatment has been proven to be safe, our previous reports attracted attention to the possibility that 60% oxygen may exacerbate ongoing acute inflammatory lung injury by inhibiting the hypoxia–HIF-1α–[adenosine]^{High}–A2AR pathway (*23*, *46*).

Therefore, it will be important to avoid using inhibitors of hypoxia–A2-adenosinergic immunosuppression, including respiratory hyperoxia, in cancer immunotherapy patients during simultaneous episodes of acute inflammation (*23*, *46*). Respiratory hyperoxia may not only enable stronger antitumor activities by de-inhibited tumor-reactive immune cells but also increase the inflammatory damage in normal tissues by de-inhibited myeloid cells or T cells activated by other antigens in patients with concomitant acute inflammation (*23*).

Because respiratory hyperoxia is widely used in clinical settings, it can be readily combined with existing immunotherapies for cancer and with already available and safe natural or synthetic antagonists of A2AR. We propose the clinical testing of respiratory hyperoxia either alone or in combination with the blockade of A2AR and inhibition of the CD39/CD73-mediated extracellular adenosine generation. It is expected that selective antagonists of A2AR will be most effective when combined with methods to reduce extracellular adenosine accumulation in the TME.

MATERIALS AND METHODS

Study design

The objective of this study was to test whether respiratory hyperoxia may prevent hypoxia-driven immunosuppression in the TME. The

tumor immunology assays in mice were expected to provide proof of principle for the potential therapeutic use of respiratory hyperoxia to overcome the inhibition of antitumor T and NK cells and improve tumor rejection. Sixty percent oxygen was selected because it is used in clinical protocols of respiratory hyperoxia. Treatment of cancer patients with advanced lung metastasis was mimicked by treatment of mice with well-established lung tumors. The maximal antitumor capacity of respiratory hyperoxia was determined by commencing treatment soon after inoculation of tumors. The MCA205 tumor cell line was used because of the predictable time course and intensity of T cell response (*30*, *31*). The more aggressive B16 melanoma was used to represent poorly immunogenic tumors (*31*, *38*). Orthotopically grown, CD73-expressing 4T1 breast tumors were also used in this study to mimic human TNBCs.

We compared 40 to 60% oxygen to provide a clinical alternative that might increase patient compliance with protocols of respiratory hyperoxia. To extend the clinical applicability, respiratory hyperoxia was combined with two major types of immunotherapy by testing whether respiratory hyperoxia would improve the preclinical therapeutic efficacy of adoptive T cell immunotherapy and dual blockade of CTLA-4 and PD-1.

Sample sizes were predetermined on the basis of statistical considerations and on pilot experiments that indicated the number of mice per group needed to generate statistical significance. Two-sided testing was performed with a confidence level of 95% for statistical analyses. Littermate mice were given tumors and randomly assigned to experimental or control groups. Where possible, treatment groups were blinded until statistical analysis. All experiments were repeated at least twice to confirm findings, and representative experiments are shown. The experimental procedures were approved by the Institutional Animal Care and Use Committee at Northeastern University.

Animals

Female C57BL/6N (B6) or Balb/c mice, 8 to 12 weeks old, were purchased from Charles River Laboratories; B6/*Thy1.1* mice were purchased from The Jackson Laboratory; γc/*Rag-2*$^{-/-}$ mice were purchased from Taconic. These animals were housed in a specific pathogen–free environment according to the National Institutes of Health (NIH) guidelines. All animal experiments were conducted in accordance with Institutional Animal Care and Use Committee guidelines of Northeastern University.

Tumors

MCA205 fibrosarcoma is a 3-methylcholanthrene–induced tumor of B6 origin (*30*, *31*), and B16-F10.P1 is a poorly immunogenic subclone of the spontaneously arising B16/BL6 melanoma (*31*, *38*). For establishment of pulmonary tumors, B6 mice were injected intravenously with either 3×10^5 MCA205 or B16-F10.P1 tumor cells suspended in 200 μl of Hanks' balanced salt solution (HBSS). On day 21, MCA205 tumor–bearing lungs were counterstained with India ink, and tumors were enumerated. Lungs with more than 250 nodules were assigned >250 as the maximum number that can be counted reliably. For survival studies, B6 mice were injected intravenously with 0.75×10^5 MCA205 tumor cells suspended in 200 μl of HBSS. For establishment of solid tumors, B6 mice were injected intradermally with 1×10^5 MCA205 tumor cells suspended in 100 μl of HBSS. For studies of spontaneous metastasis of orthotopically grown breast tumors, 1×10^5 4T1 cells (*20*, *21*) were injected into the third mammary fat pad of Balb/c mice.

Hyperoxic breathing

Mice were placed in chambers with well-controlled gas composition to mimic protocols of supplemental oxygen delivery to humans (*23*). Self-contained oxygen generators (AirSep) were used to ensure that desired levels of oxygen were maintained inside each unit. Hypercapnic acidosis was avoided by replacing traditional mouse cage tops with aerated wire lids and by using Sodasorb (Grace & Co.) (*23*, *47*, *48*). Composition of inhaled gas inside units was confirmed by analyzing PCO_2 (partial pressure of CO_2) and PO_2 (partial pressure of O_2) values in an equilibrated atmosphere. CO_2 levels inside the chamber never exceeded 0.4%, whereas hypercapnia typically occurs at levels higher than 2%. Confirmation of the levels of CO_2 and O_2 in control and experimental groups treated with 21 or 60% oxygen was done in collaboration with R. Marsh (Northeastern University). Fractional concentrations of O_2 and CO_2 were monitored by pulling a sample from the chamber at a rate of 100 ml min^{-1} using a Sable Systems Model SS3 sample pump (Sable Systems). The gas sample was pulled in order through the following: (i) a column of Drierite to remove water vapor; (ii) a Sable Systems model CA-1 CO_2 analyzer to measure the fractional concentration of CO_2; and (iii) a Sable Systems model FC-10 O_2 analyzer to measure the fractional concentration of O_2. The O_2 analyzer was calibrated using dry, CO_2-free air that was assumed to be 20.95% oxygen, and the CO_2 analyzer was calibrated using a 5.0% calibration gas from Medical-Technical Gases. Analog signals from the gas analyzers were recorded on a Macintosh computer using a 16-bit A-D converter (ADInstruments model Sp16) and the application LabChart from ADInstruments.

Monoclonal antibodies

For depletion of subsets of T and NK cells, immune cells were depleted (by intraperitoneal injection of 500 μg of either GK1.5, YTS 169, PK-136, or isotype control, Bio X Cell) 2 days before tumor inoculation and 60% oxygen treatment, preventing attack by T or NK cells on tumor cells. To maintain immune cell depletion, mAbs (250 μg) were given intraperitoneally each week until assay completion (21 days). Rat immunoglobulin G (IgG) isotype controls were given to control mice at the same dose. For CTLA-4/PD-1 dual blockade, mAbs against CTLA-4 (9H10, Bio X Cell) and PD-1 (J43, Bio X Cell) were injected (500 μg) intraperitoneally into tumor-bearing mice on days 3, 6, and 9. Mice were treated with respiratory hyperoxia from days 3 to 21 or maintained at 21% O_2 until assay completion at day 21.

Evaluation of TME hypoxia

For hypoxic localization of T cells, mice with established lung or intradermally grown MCA205 tumors were injected with Hypoxyprobe-1 (80 mg/kg). After 1.5 hours of labeling, lungs were snap-frozen, 5-μm cryosections were prepared from 10 to 20 different cutting surfaces, and immunohistochemistry was performed. For hypoxic lymphocyte analysis, mice with 11-day established MCA205 lung tumors were placed in 21 or 60% oxygen for 48 hours, and the MFI of Hypoxyprobe-1 on T cells from the lung and spleen was analyzed by flow cytometry.

Immunohistochemistry and analysis of intratumoral T cells

The infiltration of endogenous CD4/CD8 T cells into lung tumor nodules was quantified by the Harvard Medical School Pathology Department at Brigham and Women's Hospital in analyses using Spectrum Plus and Aperio's ScanScope slide scanners. Mice with 11-day established MCA205 pulmonary tumors were treated with 60% oxygen or maintained at 21% oxygen. After 4 days, the infiltration of CD4 and CD8

T cells into ~50 different lesions per group was assessed in mice breathing 21 and 60% oxygen. Immunohistochemistry was performed using 4-μm-thick acetone-fixed, optimum cutting temperature compound (OCT)–embedded tissue sections. The slides were soaked in −20°C methanol–acetic acid for 2 min and then air-dried for 20 min at room temperature. Slides were pretreated with Peroxidase Block (Dako). Primary rabbit anti-CD8 or anti-CD4 antibody (BD Pharmingen) was applied at a concentration of 1:100 at room temperature for 1 hour. Rabbit anti-rat Ig antibody was applied at a concentration of 1:750 in Dako diluent for 1 hour. Slides were detected with anti-rabbit Envision+ kit (Dako). Immunoperoxidase staining was developed using a diaminobenzidine chromogen (Dako) and counterstained with hematoxylin. Cell number per unit area was calculated after tumors were annotated using Spectrum Plus and Aperio's ScanScope slide scanners by the Harvard Medical School Pathology Department at Brigham and Women's Hospital.

For analysis of the localization of T cells in the hypoxic versus normoxic TME, tumor-bearing lungs or intradermal tumors were imbedded with OCT compound and frozen in liquid nitrogen. Sections were cut at 5 μm and mounted on glass slides. Sections were fixed in 1:1 acetone/methanol solution and stained with fluorescent-labeled Hypoxyprobe-1, CD4, and CD8 antibodies at a concentration of 1:200 for 3 hours. The slides were washed and counterstained with 4′,6-diamidino-2-phenylindole (Molecular Probes). The numbers of T cells/mm^2 in >180 hypoxic versus normoxic areas in both lung and intradermal tumors were analyzed using ImageJ software (NIH, MacBiophotonics).

Analysis of lung TME and flow cytometry

For studies of TME-infiltrating lymphocytes, mice with 11-day established MCA205 pulmonary tumors were placed in either 21 or 60% oxygen for up to 4 days. Tumor-bearing lungs were homogenized and passed through a 70-μm strainer. Lymphocytes were recovered using 40% Percoll separation, incubated with mAbs (BD Pharmingen and eBioscience) in fluorescence-activated cell sorting (FACS) buffer (phosphate-buffered saline + 0.5% bovine serum albumin), and acquired on a FACSCalibur or FACSCalibur Cytek DxP 8. Using this method, >98% of the lymphocytes were determined to be live cells by propidium iodide staining. Lymphocytes were analyzed with a lymphoid gate using CellQuest (BD Biosciences) and FlowJo (Tree Star) softwares.

Reverse transcription polymerase chain reaction

Mice with 11-day established MCA205 pulmonary tumors were placed in either 21 or 60% oxygen for 72 hours. A custom 96-well RT-PCR array was developed (Sylvester Comprehensive Cancer Center), primer sets were synthesized (Sigma), and RT-PCR was performed using the RT^2 SYBR Green PCR Mastermix (SuperArray) on an Applied Biosciences 7300 PCR platform as described previously (*35*). Housekeeping genes *Hrpt-1* and β-*actin* were used as controls. See table S2 for primer sequences.

Western blot

Mice with 11-day established lung tumors were placed in either 60 or 21% oxygen for 72 hours. Lungs were snap-frozen in liquid nitrogen and then homogenized in lysis buffer. After fractionation with SDS–polyacrylamide gel electrophoresis followed by semidry transfer, TGF-β in samples was detected with rabbit anti-mouse TGF-β polyclonal antibody conjugated to horseradish peroxidase (1:500, Santa Cruz). Levels of β-actin were detected using anti-mouse β-actin mAb (1:5000, Sigma-Aldrich).

Preparation of TDLN T cells for adoptive immunotherapy

B6 mice were inoculated subcutaneously with 1×10^6 MCA205 tumor cells in both flanks. Twelve days later, inguinal TDLNs were harvested, and single-cell suspensions were prepared and culture-activated as described previously (*30*, *31*, *49*). Four days later, TDLN cells (Fig. 7) were resuspended in HBSS for adoptive immunotherapy (*30*, *31*, *49*). Therapeutic efficacy of transferred T effector cells was assessed in the treatment of 11-day established MCA205 pulmonary tumors by intravenous injection of 5×10^6 culture-activated T cells to each mouse. Tumor-bearing mice were pretreated intravenously with cyclophosphamide (100 mg/kg) 1 day before infusion of T cells. Cyclophosphamide treatment is routinely used to improve the therapeutic efficacy of adoptively transferred T cells and was also administered to untreated tumor-bearing control mice (*38*, *49*).

Assessment of in vivo trafficking and cytokine production of tumor-reactive T cells

For fluorochrome labeling, cells were resuspended at 1×10^7/ml in HBSS containing 5 μM CFSE (Molecular Probes) as previously described (*31*). Forty-eight hours after transfer of 5×10^6 CFSE-labeled culture-activated TDLN T cells into tumor-bearing mice, lung samples were harvested and fixed in 4% formalin for 24 hours and then placed in 30% sucrose. Tissues were snap-frozen, and 5-μm cryosections were prepared from 10 to 20 different cutting surfaces. The number of CFSE-labeled cells in ~100 tumors from three mice per group was averaged and presented as the number of cells per mm^2 tumor tissue. Significance was evaluated by a Mann-Whitney test ($P = 0.01$). For cytokine analysis, TDLN T cells (5×10^6) derived from donor B6/Thy1.1$^+$ congenic mice were labeled with CFSE and injected into B6/Thy1.2$^+$ tumor-bearing recipients. Four days after transfer, Thy1.1$^+$ and Thy1.2$^+$ T cells were isolated from tumor-bearing lungs (~150 to 200 nodules), incubated with anti-CD3 (0.1 μM) for 4 hours, and analyzed by flow cytometry for the production of IFN-γ.

Statistics

The significance of differences in the numbers of pulmonary tumors between groups was measured by the Student's *t* test (two-sided), and tumor-bearing lung weights were analyzed by the Wilcoxon rank sum test. Survival studies of MCA205 tumor–bearing mice were analyzed using the log-rank test. Differences in hypoxic staining, cell numbers, RNA levels, tumor size, and flow cytometry data were analyzed by the Student's *t* test. The infiltration of transferred T cells was analyzed by the Mann-Whitney test. *P* values are listed within the figures and figure legends.

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/277/277ra30/DC1

Materials and Methods

Fig. S1. The tumor-regressing effects of respiratory hyperoxia are lost in cγ/*Rag-2*$^{-/-}$ mice.

Fig. S2. ROS scavenger does not prevent the antitumor effects of respiratory hyperoxia.

Fig. S3. Respiratory hyperoxia reverses hypoxia-adenosinergic inhibition of NK cells.

Fig. S4. Respiratory hyperoxia does not further improve the activity of tumor-reactive A2AR$^{-/-}$ T cells.

Fig. S5. CD8 and CD4 T cells avoid hypoxic TME.

Fig. S6. T_{regs} with higher expression of CTLA-4 are more hypoxic.

Fig. S7. CD8 T cells from TDLN are enriched after culture activation for adoptive transfer.
Fig. S8. Breathing 60% oxygen increased IFN-γ production by CD8 T cells in the lung TME.
Table S1. Immunostimulating cytokines/chemokines increased by respiratory hyperoxia.
Table S2. Full list of primer sets in RT-PCR arrays.

REFERENCES AND NOTES

1. A. Ohta, M. Sitkovsky, Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature* **414**, 916–920 (2001).
2. A. Ohta, E. Gorelik, S. J. Prasad, F. Ronchese, D. Lukashev, M. K. K. Wong, X. Huang, S. Caldwell, K. Liu, P. Smith, J.-F. Chen, E. K. Jackson, S. Apasov, S. Abrams, M. Sitkovsky, A2A adenosine receptor protects tumors from antitumor T cells. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 13132–13137 (2006).
3. H. K. Eltzschig, M. V. Sitkovsky, S. C. Robson, Purinergic signaling during inflammation. *N. Engl. J. Med.* **367**, 2322–2333 (2012).
4. T. Raskovalova, X. Huang, M. Sitkovsky, L. C. Zacharia, E. K. Jackson, E. Gorelik, G_s protein-coupled adenosine receptor signaling and lytic function of activated NK cells. *J. Immunol.* **175**, 4383–4391 (2005).
5. M. V. Sitkovsky, T regulatory cells: Hypoxia-adenosinergic suppression and re-direction of the immune response. *Trends Immunol.* **30**, 102–108 (2009).
6. M. V. Sitkovsky, S. Hatfield, R. Abbott, B. Belikoff, D. Lukashev, A. Ohta, Hostile, hypoxia–A2-adenosinergic tumor biology as the next barrier to overcome for tumor immunologists. *Cancer Immunol. Res.* **2**, 598–605 (2014).
7. S. A. Rosenberg, N. P. Restifo, J. C. Yang, R. A. Morgan, M. E. Dudley, Adoptive cell transfer: A clinical path to effective cancer immunotherapy. *Nat. Rev. Cancer* **8**, 299–308 (2008).
8. P. Sharma, K. Wagner, J. D. Wolchok, J. P. Allison, Novel cancer immunotherapy agents with survival benefit: Recent successes and next steps. *Nat. Rev. Cancer* **11**, 805–812 (2011).
9. J. Naidoo, D. B. Page, J. D. Wolchok, Immune modulation for cancer therapy. *Br. J. Cancer* **111**, 2214–2219 (2014).
10. I. Mellman, G. Coukos, G. Dranoff, Cancer immunotherapy comes of age. *Nature* **480**, 480–489 (2011).
11. F. S. Hodi, S. J. O'Day, D. F. McDermott, R. W. Weber, J. A. Sosman, J. B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J. C. Hassel, W. Akerley, A. J. van den Eertwegh, J. Lutzky, P. Lorigan, J. M. Vaubel, G. P. Linette, D. Hogg, C. H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J. I. Clark, J. D. Wolchok, J. S. Weber, J. Tian, M. J. Yellin, G. M. Nichol, A. Hoos, W. J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
12. D. M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy. *Nat. Rev. Cancer* **12**, 252–264 (2012).
13. A. Young, D. Mittal, J. Stagg, M. J. Smyth, Targeting cancer-derived adenosine: New therapeutic approaches. *Cancer Discov.* **4**, 879–888 (2014).
14. C. M. Koebel, W. Vermi, J. B. Swann, N. Zerafa, S. J. Rodig, L. J. Old, M. J. Smyth, R. D. Schreiber, Adaptive immunity maintains occult cancer in an equilibrium state. *Nature* **450**, 903–907 (2007).
15. A. T. Waickman, A. Alme, L. Senaldi, P. E. Zarek, M. Horton, J. D. Powell, Enhancement of tumor immunotherapy by deletion of the A_{2A} adenosine receptor. *Cancer Immunol. Immunother.* **61**, 917–926 (2012).
16. B. M. Künzli, M. I. Bernlochner, S. Rath, S. Käser, E. Csizmadia, K. Enjyoji, P. Cowan, A. d'Apice, K. Dwyer, R. Rosenberg, A. Perren, H. Friess, C. A. Maurer, S. C. Robson, Impact of CD39 and purinergic signalling on the growth and metastasis of colorectal cancer. *Purinergic Signal.* **7**, 231–241 (2011).
17. J. Stagg, U. Divisekera, N. McLaughlin, J. Sharkey, S. Pommey, D. Denoyer, K. M. Dwyer, and M. J. Smyth, Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 1547–1552 (2010).
18. J. Stagg, U. Divisekera, H. Duret, T. Sparwasser, M. W. L. Teng, P. K. Darcy, M. J. Smyth, CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. *Cancer Res.* **71**, 2892–2900 (2011).
19. D. Jin, J. Fan, L. Wang, L. F. Thompson, A. Liu, B. J. Daniel, T. Shin, T. J. Curiel, B. Zhang, CD73 on tumor cells impairs antitumor T-cell responses: A novel mechanism of tumor-induced immune suppression. *Cancer Res.* **70**, 2245–2255 (2010).
20. P. A. Beavis, U. Divisekera, C. Paget, M. T. Chow, L. B. John, C. Devaud, K. Dwyer, J. Stagge, M. J. Smyth, P. K. Darcy, Blockade of A_{2A} receptors potently suppresses the metastasis of $CD73^+$ tumors. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 14711–14716 (2013).
21. S. Loi, S. Pommey, B. Haibe-Kains, P. A. Beavis, P. K. Darcy, M. J. Smyth, J. Stagg, CD73 promotes anthracycline resistance and poor prognosis in triple negative breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 11091–11096 (2013).
22. R. H. Kallet, M. A. Matthay, Hyperoxic acute lung injury. *Respir. Care* **58**, 123–141 (2013).
23. M. Thiel, A. Chouker, A. Ohta, E. Jackson, C. Caldwell, P. Smith, D. Lukashev, I. Bittmann, M. V. Sitkovsky, Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. *PLOS Biol.* **3**, e174 (2005).
24. P. Vaupel, Tumor oxygenation: An appraisal of past and present concepts and a look into the future: Arisztid G. B. Kovách Lecture. *Adv. Exp. Med. Biol.* **789**, 229–236 (2013).
25. S. M. Hatfield, J. Kjaergaard, D. Lukashev, B. Belikoff, T. H. Schreiber, S. Sethumadhavan, R. Abbott, P. Philbrook, M. Thayer, D. Shujia, S. Rodig, J. L. Kutok, J. Ren, A. Ohta, E. R. Podack, B. Karger, E. K. Jackson, M. Sitkovsky, Systemic oxygenation weakens the hypoxia and hypoxia inducible factor 1α-dependent and extracellular adenosine-mediated tumor protection. *J. Mol. Med.* **92**, 1283–1292 (2014).
26. M. W. Dewhirst, Y. Cao, B. Moeller, Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. *Nat. Rev. Cancer* **8**, 425–437 (2008).
27. J. Wang, J. Yi, Cancer cell killing via ROS: To increase or decrease, that is the question. *Cancer Biol. Ther.* **7**, 1875–1884 (2008).
28. J. H. Min, C. N. Codipilly, S. Nasim, E. J. Miller, M. N. Ahmed, Synergistic protection against hyperoxia-induced lung injury by neutrophils blockade and EC-SOD overexpression. *Respir. Res.* **13**, 58 (2012).
29. E. Kratzer, Y. Tian, N. Sarich, T. Wu, A. Meliton, A. Leff, A. A. Birukova, Oxidative stress contributes to lung injury and barrier dysfunction via microtubule destabilization. *Am. J. Respir. Cell Mol. Biol.* **47**, 688–697 (2012).
30. J. Kjaergaard, S. Shu, Tumor infiltration by adoptively transferred T cells is independent of immunologic specificity but requires down-regulation of L-selectin expression. *J. Immunol.* **163**, 751–759 (1999).
31. J. Kjaergaard, L. Peng, P. A. Cohen, S. Shu, Therapeutic efficacy of adoptive immunotherapy is predicated on in vivo antigen-specific proliferation of donor T cells. *Clin. Immunol.* **108**, 8–20 (2003).
32. M. Champsaur, L. L. Lanier, Effect of NKG2D ligand expression on host immune responses. *Immunol. Rev.* **235**, 267–285 (2010).
33. D. H. Raulet, S. Gasser, B. G. Gowen, W. Deng, H. Jung, Regulation of ligands for the NKG2D activating receptor. *Annu. Rev. Immunol.* **31**, 413–441 (2013).
34. S. A. Quezada, K. S. Peggs, T. R. Simpson, Y. Shen, D. R. Littman, J. P. Allison, Limited tumor infiltration by activated T effector cells restricts the therapeutic activity of regulatory T cell depletion against established melanoma. *J. Exp. Med.* **205**, 2125–2138 (2008).
35. T. H. Schreiber, V. V. Deyev, J. D. Rosenblatt, E. R. Podack, Tumor-induced suppression of CTL expansion and subjugation by gp96-Ig vaccination. *Cancer Res.* **69**, 2026–2033 (2009).
36. M. V. Sitkovsky, J. Kjaergaard, D. Lukashev, A. Ohta, Hypoxia-adenosinergic immunosuppression: Tumor protection by T regulatory cells and cancerous tissue hypoxia. *Clin. Cancer Res.* **14**, 5947–5952 (2008).
37. K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, M. Miyara, Z. Fehervari, T. Nomura, S. Sakaguchi, CTLA-4 control over $Foxp3^+$ regulatory T cell function. *Science* **322**, 271–275 (2008).
38. J. Kjaergaard, L. Peng, P. A. Cohen, J. A. Drazba, A. D. Weinberg, S. Shu, Augmentation versus inhibition: Effects of conjunctional OX-40 receptor monoclonal antibody and IL-2 treatment on adoptive immunotherapy of advanced tumor. *J. Immunol.* **167**, 6669–6677 (2001).
39. M. Koshiba, H. Kojima, S. Huang, S. Apasov, M. V. Sitkovsky, Memory of extracellular adenosine A_{2A} purinergic receptor-mediated signaling in murine T cells. *J. Biol. Chem.* **272**, 25881–25889 (1997).
40. S. Kobayashi, H. Zimmermann, D. E. Millhorn, Chronic hypoxia enhances adenosine release in rat PC12 cells by altering adenosine metabolism and membrane transport. *J. Neurochem.* **74**, 621–632 (2000).
41. U. K. Decking, G. Schlieper, K. Kroll, J. Schrader, Hypoxia-induced inhibition of adenosine-kinase potentiates cardiac adenosine release. *Circ. Res.* **81**, 154–164 (1997).
42. K. Synnestvedt, G. T. Furuta, K. M. Comerford, N. Louis, J. Karhausen, H. K. Eltzschig, K. R. Hansen, L. F. Thompson, S. P. Colgan, Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J. Clin. Invest.* **110**, 993–1002 (2002).
43. C. A. Corzo, T. Condamine, L. Lu, M. J. Cotter, J. I. Youn, P. Cheng, H. I. Cho, E. Celis, D. G. Quiceno, T. Padhya, T. V. McCaffrey, J. C. McCaffrey, D. I. Gabrilovich, HIF-1α regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J. Exp. Med.* **207**, 2439–2453 (2010).
44. O. R. Colegio, N.-Q. Chu, A. L. Szabo, T. Chu, A. Marie Rhebergen, V. Jairam, N. Cyrus, C. E. Brokowski, S. C. Eisenbarth, G. M. Phillips, G. W. Cline, A. J. Phillips, R. Medzhitov, Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* **513**, 559–563 (2014).
45. P. Chaturvedi, D. M. Gilkes, N. Takano, G. L. Semenza, Hypoxia-inducible factor-dependent signaling between triple-negative breast cancer cells and mesenchymal stem cells promotes macrophage recruitment. *Proc. Natl. Acad. Sci. U.S.A.* **111**, E2120–E2129 (2014).
46. A. Ohta, M. Sitkovsky, Caveats in promising therapeutic targeting of the anti-inflammatory A2 adenosine receptors: The notes of caution. *Nat. Rev. Drug Discov.* **5** (2006).
47. H. Ooi, E. Cadogan, M. Sweeney, K. Howell, R. G. O'Regan, P. McLoughlin, Chronic hypercapnia inhibits hypoxic pulmonary vascular remodeling. *Am. J. Physiol. Heart Circ. Physiol.* **278**, H331–H338 (2000).

48. H. R. De Smet, A. D. Bersten, H. A. Barr, I. R. Doyle, Hypercapnic acidosis modulates inflammation, lung mechanics, and edema in the isolated perfused lung. *J. Crit. Care* **22**, 305–313 (2007).
49. J. Kjaergaard, J. Tanaka, J. A. Kim, K. Rothchild, A. Weinberg, S. Shu, Therapeutic efficacy of OX-40 receptor antibody depends on tumor immunogenicity and anatomic site of tumor growth. *Cancer Res.* **60**, 5514–5521 (2000).

Acknowledgments: We thank J. Stagg at the University of Montreal for providing the 4T1 tumor cell line and sharing his expertise. We thank S. Ohman for assistance in all steps leading to the preparation of the manuscript. We also thank R. Marsh, professor of biology at Northeastern University, for assistance with monitoring and controlling gas levels in hyperoxic units. **Funding:** This work was supported by funding from Northeastern University and NIH grants to M.V.S. (R01 CA 112561, R01 CA 111985, R21 AT 002788, U19 AI 091693, Dana-Farber Cancer Institute, and Harvard Medical School–Northeastern University Joint Program in Cancer Drug Development) as well as by National Cancer Institute grant 5PO1CA109094-03 and National Institute of Allergy and Infectious Diseases 1P01 grant AI096396-01 3 to E.R.P.; HL109002, DK091190, DK068575, DK079307, and CA168628 to E. K. J.; and a Bankhead-Coley Postdoctoral Fellowship to T.H.S. **Author contributions:** S.M.H., J.K., A.O., and M.V.S. performed and/or analyzed cancer immunology assays. S.R. and J.L.K. performed, enumerated, and interpreted immunohistochemistry assays. J.K. established and supervised lung tumor models. E.R.P. and T.H.S. designed and performed assays with custom-made RNA arrays to scan lung TME. D.L., B.B., R.A., S.S., P.P., K.K., R.C., and M.T. performed or assisted in tumor immunology, immunohistochemistry, and flow cytometry assays. E.K.J. and B.K. analyzed and interpreted changes in the TME. M.V.S. designed the overall approach, directed all stages of research, and wrote the manuscript with S.M.H. **Competing interests:** U.S. government, NIH holds an issued patent "Methods for using extracellular adenosine inhibitors and adenosine receptor inhibitors to enhance immune response and inflammation," US 8,716,301, which is related to the work described in this paper, with A.O. and M.V.S. named as inventors. M.V.S. is a founder of RedoxTherapies, a company that is charged with the translation of this approach into the clinic and has licensed this patent. E.R.P. has a provisional patent for "Anti-immune suppression tumor therapy" and is the scientific cofounder, paid consultant, and equity owner of Heat Biologics Inc., and T.H.S. is an employee at Heat Biologics Inc. J.L.K. is an employee and shareholder at Infinity Pharmaceuticals Inc. E.K.J. holds equity in Adenopaint, which is unrelated to the current study. **Data and materials availability:** Correspondence and requests for materials should be addressed to M.V.S. (m.sitkovsky@neu.edu).

Submitted 23 October 2014
Accepted 28 January 2015
Published 4 March 2015
10.1126/scitranslmed.aaa1260

Citation: S. M. Hatfield, J. Kjaergaard, D. Lukashev, T. H. Schreiber, B. Belikoff, R. Abbott, S. Sethumadhavan, P. Philbrook, K. Ko, R. Cannici, M. Thayer, S. Rodig, J. L. Kutok, E. K. Jackson, B. Karger, E. R. Podack, A. Ohta, M. V. Sitkovsky, Immunological mechanisms of the antitumor effects of supplemental oxygenation. *Sci. Transl. Med.* **7**, 277ra30 (2015).

Science Translational Medicine **publishes original research articles and reports that represent significant movements toward improving human health, including new biology and advances in the diagnosis and treatment of disease.**

CANCER

On being less tolerant: Enhanced cancer immunosurveillance enabled by targeting checkpoints and agonists of T cell activation

Alexander M. Lesokhin *et al.* (Jedd Wolchok)

Citation
Sci. Transl. Med. 25 Mar 2015: Vol. 7, Issue 280, pp. 280sr1

10.1126/scitranslmed.3010274

The recent approvals of two drugs that block the function of the immune checkpoint programmed cell death 1 (PD-1) have firmly planted tumor immunotherapy in the mainstream of clinical oncology.

CANCER

Delineating cancer evolution with single-cell sequencing

Nicholas E. Navin

Citation
Sci. Transl. Med. 15 Jul 2015: Vol. 7, Issue 296, pp. 296fs29

10.1126/scitranslmed.aac8319

Single-cell sequencing methods are revolutionizing cancer research and medicine by providing powerful tools to investigate intratumor heterogeneity and rare subpopulations.

IMMUNOTHERAPY

Adoptive cellular therapy: A race to the finish line

Carl H. June, Stanley R. Riddell, and Ton N. Schumacher

Citation
Sci. Transl. Med. 25 Mar 2015: Vol. 7, Issue 280, pp. 280ps7

10.1126/scitranslmed.aaa3643

Adoptive T cell transfer for cancer, chronic infection, and autoimmunity is an emerging field that shows promise in recent trials.

IMMUNOLOGY

Hurdles in therapy with regulatory T cells

Piotr Trzonkowski *et al.*

Citation
Sci. Transl. Med. 09 Sep 2015: Vol. 7, Issue 304, pp. 304ps18

10.1126/scitranslmed.aaa7721

Improper activation of the immune system contributes to a variety of clinical conditions, including autoimmune and allergic diseases as well as solid organ and bone marrow transplantation.

Science Translational Medicine **publishes original research articles that report promising advances toward improving human health, including new biology and advances in the diagnosis and treatment of disease.**

CANCER

Antioxidants can increase melanoma metastasis in mice

Kristell Le Gal *et al.* (Martin Bergo)

Citation
Sci. Transl. Med. 07 Oct 2015: Vol. 7, Issue 308, pp. 308re8

10.1126/scitranslmed.aad3740

Antioxidants in the diet and supplements are widely used to protect against cancer, but clinical trials with antioxidants do not support this concept. Some trials show that antioxidants actually increase cancer risk and a study in mice showed that antioxidants accelerate the progression of primary lung tumors. However, little is known about the impact of antioxidant supplementation on the progression of other types of cancer, including malignant melanoma. We show that administration of *N*-acetylcysteine (NAC) increases lymph node metastases in an endogenous mouse model of malignant melanoma but has no impact on the number and size of primary tumors. Similarly, NAC and the soluble vitamin E analog Trolox markedly increased the migration and invasive properties of human malignant melanoma cells but did not affect their proliferation. Both antioxidants increased the ratio between reduced and oxidized glutathione in melanoma cells and in lymph node metastases, and the increased migration depended on new glutathione synthesis. Furthermore, both NAC and Trolox increased the activation of the small guanosine triphosphatase (GTPase) RHOA, and blocking downstream RHOA signaling abolished antioxidant-induced migration. These results demonstrate that antioxidants and the glutathione system play a previously unappreciated role in malignant melanoma progression.

CANCER

Therapeutic targeting of the MYC signal by inhibition of histone chaperone FACT in neuroblastoma

Daniel Carter *et al.* (Glenn Marshall)

Citation
Sci. Transl. Med. 04 Nov 2015: Vol. 7, Issue 312, pp. 312ra176

10.1126/scitranslmed.aab1803

Amplification of the *MYCN* oncogene predicts treatment resistance in childhood neuroblastoma. We used a MYC target gene signature that predicts poor neuroblastoma prognosis to identify the histone chaperone FACT (facilitates chromatin transcription) as a crucial mediator of the MYC signal and a therapeutic target in the disease. FACT and MYCN expression created a forward feedback loop in neuroblastoma cells that was essential for maintaining mutual high expression. FACT inhibition by the small-molecule curaxin compound CBL0137 markedly reduced tumor initiation and progression in vivo. CBL0137 exhibited strong synergy with standard chemotherapy by blocking repair of DNA damage caused by genotoxic drugs, thus creating a synthetic lethal environment in *MYCN*-amplified neuroblastoma cells and suggesting a treatment strategy for MYCN-driven neuroblastoma.

CANCER

Plasma *AR* and abiraterone-resistant prostate cancer

Alessandro Romanel *et al.* (Gerhardt Attard)

Citation
Sci. Transl. Med. 04 Nov 2015: Vol. 7, Issue 312, pp. 312re10

10.1126/scitranslmed.aac9511

Androgen receptor (*AR*) gene aberrations are rare in prostate cancer before primary hormone treatment but emerge with castration resistance. To determine *AR* gene status using a minimally invasive assay that could have broad clinical utility, we developed a targeted next-generation sequencing approach amenable to plasma DNA, covering all AR coding bases and genomic regions that are highly informative in prostate cancer. We sequenced 274 plasma samples from 97 castration-resistant prostate cancer patients treated with abiraterone at two institutions. We controlled for normal DNA in patients' circulation and detected a sufficiently high tumor DNA fraction to quantify *AR* copy number state in 217 samples (80 patients). Detection of *AR* copy number gain and point mutations in plasma were inversely correlated, supported further by the enrichment of nonsynonymous versus synonymous mutations in *AR* copy number normal as opposed to *AR* gain samples. Whereas *AR* copy number was unchanged from before treatment to progression and no mutant *AR* alleles showed signal for acquired gain, we observed emergence of T878A or L702H AR amino acid changes in 13% of tumors at progression on abiraterone. Patients with *AR* gain or T878A or L702H before abiraterone (45%) were 4.9 and 7.8 times less likely to have a ≥50 or ≥90% decline in prostate-specific antigen (PSA), respectively, and had a significantly worse overall [hazard ratio (HR), 7.33; 95% confidence interval (CI), 3.51 to 15.34; $P = 1.3 \times 10^{-9}$) and progression-free (HR, 3.73; 95% CI, 2.17 to 6.41; $P = 5.6 \times 10^{-7}$) survival. Evaluation of plasma *AR* by next-generation sequencing could identify cancers with primary resistance to abiraterone.

CANCER

Castration radiosensitizes prostate cancer tissue by impairing DNA double-strand break repair

Firas Tarish *et al.* (Thomas Helleday)

Citation
Sci. Transl. Med. 04 Nov 2015: Vol. 7, Issue 312, pp. 312re11

10.1126/scitranslmed.aac5671

Chemical castration improves responses to radiotherapy in prostate cancer, but the mechanism is unknown. We hypothesized that this radiosensitization is caused by castration-mediated down-regulation of nonhomologous end joining (NHEJ) repair of DNA double-strand breaks (DSBs). To test this, we enrolled 48 patients with localized prostate cancer in two arms of the study: either radiotherapy first or radiotherapy after neoadjuvant castration treatment. We biopsied patients at diagnosis and before and after castration and radiotherapy treatments to monitor androgen receptor, NHEJ, and DSB repair in verified cancer tissue. We show that patients receiving neoadjuvant castration treatment before radiotherapy had reduced amounts of the NHEJ protein Ku70, impaired radiotherapy-induced NHEJ activity, and higher amounts of unrepaired DSBs, measured by γ-H2AX foci in cancer tissues. This study demonstrates that chemical castration impairs NHEJ activity in prostate cancer tissue, explaining the improved response of patients with prostate cancer to radiotherapy after chemical castration.

CANCER

Targeting LGR5+ cells with an antibody-drug conjugate for the treatment of colon cancer

Melissa R. Junttila *et al.* (Andrew G. Polson)

Citation
Sci. Transl. Med. 18 Nov 2015:
Vol. 7, Issue 314, pp. 314ra186

10.1126/scitranslmed.aac7433

Cancer stem cells (CSCs) are hypothesized to actively maintain tumors similarly to how their normal counterparts replenish differentiated cell types within tissues, making them an attractive therapeutic target for the treatment of cancer. Because most CSC markers also label normal tissue stem cells, it is unclear how to selectively target them without compromising normal tissue homeostasis. We evaluated a strategy that targets the cell surface leucine-rich repeat–containing G protein–coupled receptor 5 (LGR5), a well-characterized tissue stem cell and CSC marker, with an antibody conjugated to distinct cytotoxic drugs. One antibody-drug conjugate (ADC) demonstrated potent tumor efficacy and safety in vivo. Furthermore, the ADC decreased tumor size and proliferation, translating to improved survival in a genetically engineered model of intestinal tumorigenesis. These data demonstrate that ADCs can be leveraged to exploit differences between normal and cancer stem cells to successfully target gastrointestinal cancers.

CANCER

AZD9150, a next-generation antisense oligonucleotide inhibitor of *STAT3* with early evidence of clinical activity in lymphoma and lung cancer

David Hong *et al.* (A. Robert MacLeod)

Citation
Sci. Transl. Med. 18 Nov 2015:
Vol. 7, Issue 314, pp. 314ra185

10.1126/scitranslmed.aac5272

Next-generation sequencing technologies have greatly expanded our understanding of cancer genetics. Antisense technology is an attractive platform with the potential to translate these advances into improved cancer therapeutics, because antisense oligonucleotide (ASO) inhibitors can be designed on the basis of gene sequence information alone. Recent human clinical data have demonstrated the potent activity of systemically administered ASOs targeted to genes expressed in the liver. We describe the preclinical activity and initial clinical evaluation of a class of ASOs containing constrained ethyl modifications for targeting the gene encoding the transcription factor STAT3, a notoriously difficult protein to inhibit therapeutically. Systemic delivery of the unformulated ASO, AZD9150, decreased *STAT3* expression in a broad range of preclinical cancer models and showed antitumor activity in lymphoma and lung cancer models. AZD9150 preclinical activity translated into single-agent antitumor activity in patients with highly treatment-refractory lymphoma and non–small cell lung cancer in a phase 1 dose-escalation study.

CANCER

STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade

Juan Fu *et al.* (Young Kim)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra52

10.1126/scitranslmed.aaa4306

Stimulator of interferon genes (STING) is a cytosolic receptor that senses both exogenous and endogenous cytosolic cyclic dinucleotides (CDNs), activating TBK1/IRF3 (interferon regulatory factor 3), NF-κB (nuclear factor κB), and STAT6 (signal transducer and activator of transcription 6) signaling pathways to induce robust type I interferon and proinflammatory cytokine responses. CDN ligands were formulated with granulocyte-macrophage colony-stimulating factor (GM-CSF)–producing cellular cancer vaccines—termed STINGVAX—that demonstrated potent in vivo antitumor efficacy in multiple therapeutic models of established cancer. We found that rationally designed synthetic CDN derivative molecules, including one with an Rp,Rp dithio diastereomer and noncanonical c[A(2′,5′)pA(3′,5′)p] phosphate bridge structure, enhanced antitumor efficacy of STINGVAX in multiple aggressive therapeutic models of established cancer in mice. Antitumor activity was STING-dependent and correlated with increased activation of dendritic cells and tumor antigen–specific $CD8^+$ T cells. Tumors from STINGVAX-treated mice demonstrated marked PD-L1 (programmed death ligand 1) up-regulation, which was associated with tumor-infiltrating $CD8^+IFN\gamma^+$ T cells. When combined with PD-1 (programmed death 1) blockade, STINGVAX induced regression of palpable, poorly immunogenic tumors that did not respond to PD-1 blockade alone.

CANCER

Clonal status of actionable driver events and the timing of mutational processes in cancer evolution

Nicholas McGranahan *et al.* (Charles Swanton)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra54

10.1126/scitranslmed.aaa1408

Deciphering whether actionable driver mutations are found in all or a subset of tumor cells will likely be required to improve drug development and precision medicine strategies. We analyzed nine cancer types to determine the subclonal frequencies of driver events, to time mutational processes during cancer evolution, and to identify drivers of subclonal expansions. Although mutations in known driver genes typically occurred early in cancer evolution, we also identified later subclonal "actionable" mutations, including *BRAF*(V600E), *IDH1* (R132H), *PIK3CA* (E545K), *EGFR* (L858R), and *KRAS* (G12D), which may compromise the efficacy of targeted therapy approaches. More than 20% of *IDH1*mutations in glioblastomas, and 15% of mutations in genes in the PI3K (phosphatidylinositol 3-kinase)–AKT–mTOR (mammalian target of rapamycin) signaling axis across all tumor types were subclonal. Mutations in the RAS–MEK (mitogen-activated protein kinase kinase) signaling axis were less likely to be subclonal than mutations in genes associated with PI3K-AKT-mTOR signaling. Analysis of late mutations revealed a link between APOBEC-mediated mutagenesis and the acquisition of subclonal driver mutations and uncovered putative cancer genes involved in subclonal expansions, including *CTNNA2* and *ATXN1*. Our results provide a pan-cancer census of driver events within the context of intratumor heterogeneity and reveal patterns of tumor evolution across cancers. The frequent presence of subclonal driver mutations suggests the need to stratify targeted therapy response according to the proportion of tumor cells in which the driver is identified.

CANCER

Tumor cells, but not endothelial cells, mediate eradication of primary sarcomas by stereotactic body radiation therapy

Everett J. Moding *et al.* (David Kirsch)

Citation
Sci. Transl. Med. 11 Mar 2015: Vol. 7, Issue 278, pp. 278ra34

10.1126/scitranslmed.aaa4214

Cancer clinics currently use high-dose stereotactic body radiation therapy as a curative treatment for several kinds of cancers. However, the contribution of vascular endothelial cells to tumor response to radiation remains controversial. Using dual recombinase technology, we generated primary sarcomas in mice with targeted genetic mutations specifically in tumor cells or endothelial cells. We selectively mutated the proapoptotic gene *Bax* or the DNA damage response gene *Atm* to genetically manipulate the radiosensitivity of endothelial cells in primary soft tissue sarcomas. *Bax* deletion from endothelial cells did not affect radiation-induced cell death in tumor endothelial cells or sarcoma response to radiation therapy. Although Atm deletion increased endothelial cell death after radiation therapy, deletion of *Atm* from endothelial cells failed to enhance sarcoma eradication. In contrast, deletion of *Atm* from tumor cells increased sarcoma eradication by radiation therapy. These results demonstrate that tumor cells, rather than endothelial cells, are critical targets that regulate sarcoma eradication by radiation therapy. Treatment with BEZ235, a small-molecule protein kinase inhibitor, radiosensitized primary sarcomas more than the heart. These results suggest that inhibiting ATM kinase during radiation therapy is a viable strategy for radiosensitization of some tumors.

CANCER

PI3K inhibition results in enhanced estrogen receptor function and dependence in hormone receptor–positive breast cancer

Ana Bosch *et al.* (Jose Baselga)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra51

10.1126/scitranslmed.aaa4442

Activating mutations of PIK3CA are the most frequent genomic alterations in estrogen receptor (ER)–positive breast tumors, and selective phosphatidylinositol 3-kinase α (PI3Kα) inhibitors are in clinical development. The activity of these agents, however, is not homogeneous, and only a fraction of patients bearing PIK3CA-mutant ER-positive tumors benefit from single-agent administration. Searching for mechanisms of resistance, we observed that suppression of PI3K signaling results in induction of ER-dependent transcriptional activity, as demonstrated by changes in expression of genes containing ER-binding sites and increased occupancy by the ER of promoter regions of up-regulated genes. Furthermore, expression of ESR1 mRNA and ER protein were also increased upon PI3K inhibition. These changes in gene expression were confirmed in vivo in xenografts and patient-derived models and in tumors from patients undergoing treatment with the PI3Kα inhibitor BYL719. The observed effects on transcription were enhanced by the addition of estradiol and suppressed by the anti-ER therapies fulvestrant and tamoxifen. Fulvestrant markedly sensitized ER-positive tumors to PI3Kα inhibition, resulting in major tumor regressions in vivo. We propose that increased ER transcriptional activity may be a reactive mechanism that limits the activity of PI3K inhibitors and that combined PI3K and ER inhibition is a rational approach to target these tumors.

CANCER

Personalized genomic analyses for cancer mutation discovery and interpretation

Siân Jones *et al.* (Luis Diaz Jr. and Victor Velculescu)

Citation
Sci. Transl. Med. 15 Apr 2015: Vol. 7, Issue 283, pp. 283ra53

10.1126/scitranslmed.aaa7161

Massively parallel sequencing approaches are beginning to be used clinically to characterize individual patient tumors and to select therapies based on the identified mutations. A major question in these analyses is the extent to which these methods identify clinically actionable alterations and whether the examination of the tumor tissue alone is sufficient or whether matched normal DNA should also be analyzed to accurately identify tumor-specific (somatic) alterations. To address these issues, we comprehensively evaluated 815 tumor-normal paired samples from patients of 15 tumor types. We identified genomic alterations using next-generation sequencing of whole exomes or 111 targeted genes that were validated with sensitivities >95% and >99%, respectively, and specificities >99.99%. These analyses revealed an average of 140 and 4.3 somatic mutations per exome and targeted analysis, respectively. More than 75% of cases had somatic alterations in genes associated with known therapies or current clinical trials. Analyses of matched normal DNA identified germline alterations in cancer-predisposing genes in 3% of patients with apparently sporadic cancers. In contrast, a tumor-only sequencing approach could not definitively identify germline changes in cancer-predisposing genes and led to additional false-positive findings comprising 31% and 65% of alterations identified in targeted and exome analyses, respectively, including in potentially actionable genes. These data suggest that matched tumor-normal sequencing analyses are essential for precise identification and interpretation of somatic and germline alterations and have important implications for the diagnostic and therapeutic management of cancer patients.

CANCER

Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck SCC

Yuxuan Wang *et al.* (Ken Kinzler, Bert Vogelstein, and Nishant Agrawal)

Citation
Sci. Transl. Med. 24 Jun 2015: Vol. 7, Issue 293, pp. 293ra104

10.1126/scitranslmed.aaa8507

To explore the potential of tumor-specific DNA as a biomarker for head and neck squamous cell carcinomas (HNSCC), we queried DNA from saliva or plasma of 93 HNSCC patients. We searched for somatic mutations or human papillomavirus genes, collectively referred to as tumor DNA. When both plasma and saliva were tested, tumor DNA was detected in 96% of 47 patients. The fractions of patients with detectable tumor DNA in early- and late-stage disease were 100% (n = 10) and 95% (n = 37), respectively. When segregated by site, tumor DNA was detected in 100% (n = 15), 91% (n = 22), 100% (n = 7), and 100% (n = 3) of patients with tumors of the oral cavity, oropharynx, larynx, and hypopharynx, respectively. In saliva, tumor DNA was found in 100% of patients with oral cavity cancers and in 47 to 70% of patients with cancers of the other sites. In plasma, tumor DNA was found in 80% of patients with oral cavity cancers, and in 86 to 100% of patients with cancers of the other sites. Thus, saliva is preferentially enriched for tumor DNA from the oral cavity, whereas plasma is preferentially enriched for tumor DNA from the other sites. Tumor DNA in saliva was found postsurgically in three patients before clinical diagnosis of recurrence, but in none of the five patients without recurrence. Tumor DNA in the saliva and plasma appears to be a potentially valuable biomarker for detection of HNSCC.

CANCER

Magnetic resonance image features identify glioblastoma phenotypic subtypes with distinct molecular pathway activities

Haruka Itakura *et al.* (Olivier Gevaert)

Citation
Sci. Transl. Med. 02 Sep 2015: Vol. 7, Issue 303, pp. 303ra138

10.1126/scitranslmed.aaa7582

Glioblastoma (GBM) is the most common and highly lethal primary malignant brain tumor in adults. There is a dire need for easily accessible, noninvasive biomarkers that can delineate underlying molecular activities and predict response to therapy. To this end, we sought to identify subtypes of GBM, differentiated solely by quantitative magnetic resonance (MR) imaging features, that could be used for better management of GBM patients. Quantitative image features capturing the shape, texture, and edge sharpness of each lesion were extracted from MR images of 121 single-institution patients with de novo, solitary, unilateral GBM. Three distinct phenotypic "clusters" emerged in the development cohort using consensus clustering with 10,000 iterations on these image features. These three clusters—pre-multifocal, spherical, and rim-enhancing, names reflecting their image features—were validated in an independent cohort consisting of 144 multi-institution patients with similar tumor characteristics from The Cancer Genome Atlas (TCGA). Each cluster mapped to a unique set of molecular signaling pathways using pathway activity estimates derived from the analysis of TCGA tumor copy number and gene expression data with the PARADIGM (Pathway Recognition Algorithm Using Data Integration on Genomic Models) algorithm. Distinct pathways, such as c-Kit and FOXA, were enriched in each cluster, indicating differential molecular activities as determined by the image features. Each cluster also demonstrated differential probabilities of survival, indicating prognostic importance. Our imaging method offers a noninvasive approach to stratify GBM patients and also provides unique sets of molecular signatures to inform targeted therapy and personalized treatment of GBM.

IMMUNOTHERAPY

Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia

David L. Porter *et al.* (Carl June)

Citation
Sci. Transl. Med. 02 Sep 2015: Vol. 7, Issue 303, pp. 303ra139

110.1126/scitranslmed.aac5415

Patients with multiply relapsed or refractory chronic lymphocytic leukemia (CLL) have a poor prognosis. Chimeric antigen receptor (CAR)–modified T cells targeting CD19 have the potential to improve on the low complete response rates with conventional therapies by inducing sustained remissions in patients with refractory B cell malignancies. We previously reported preliminary results on three patients with refractory CLL. We report the mature results from our initial trial using CAR-modified T cells to treat 14 patients with relapsed and refractory CLL. Autologous T cells transduced with a CD19-directed CAR (CTL019) lentiviral vector were infused into patients with relapsed/refractory CLL at doses of 0.14×10^8 to 11×10^8 CTL019 cells (median, 1.6×10^8 cells). Patients were monitored for toxicity, response, expansion, and persistence of circulating CTL019 T cells. The overall response rate in these heavily pretreated CLL patients was 8 of 14 (57%), with 4 complete remissions (CR) and 4 partial remissions (PR). The in vivo expansion of the CAR T cells correlated with clinical responses, and the CAR T cells persisted and remained functional beyond 4 years in the first two patients achieving CR. No patient in CR has relapsed. All responding patients developed B cell aplasia and experienced cytokine release syndrome, coincident with T cell proliferation. Minimal residual disease was not detectable in patients who achieved CR, suggesting that disease eradication may be possible in some patients with advanced CLL.

CANCER

Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer

Peter van Luijk *et al.* (Robert Coppes)

Citation
Sci. Transl. Med. 16 Sep 2015:
Vol. 7, Issue 305, pp. 305ra147

10.1126/scitranslmed.aac4441

Each year, 500,000 patients are treated with radiotherapy for head and neck cancer, resulting in relatively high survival rates. However, in 40% of patients, quality of life is severely compromised because of radiation-induced impairment of salivary gland function and consequent xerostomia (dry mouth). New radiation treatment technologies enable sparing of parts of the salivary glands. We have determined the parts of the major salivary gland, the parotid gland, that need to be spared to ensure that the gland continues to produce saliva after irradiation treatment. In mice, rats, and humans, we showed that stem and progenitor cells reside in the region of the parotid gland containing the major ducts. We demonstrated in rats that inclusion of the ducts in the radiation field led to loss of regenerative capacity, resulting in long-term gland dysfunction with reduced saliva production. Then we showed in a cohort of patients with head and neck cancer that the radiation dose to the region of the salivary gland containing the stem/progenitor cells predicted the function of the salivary glands one year after radiotherapy. Finally, we showed that this region of the salivary gland could be spared during radiotherapy, thus reducing the risk of post-radiotherapy xerostomia.

A selection of graphical abstracts that provide a visual summary of the key points of a research article.

CANCER

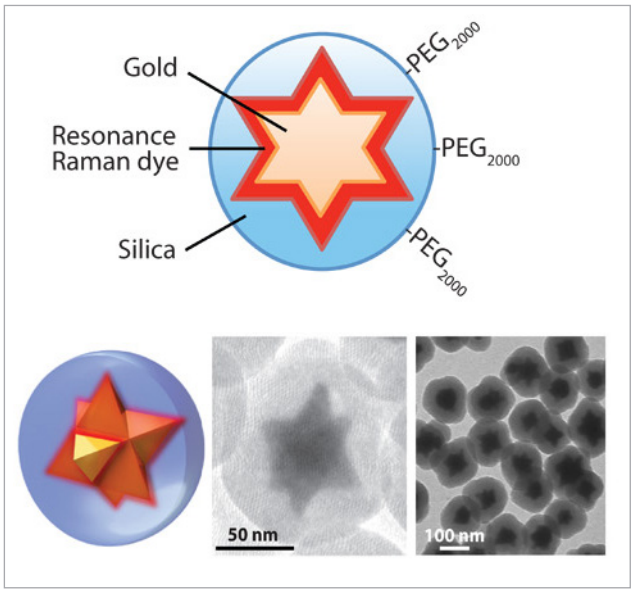
Surface-enhanced resonance Raman scattering nanostars for high-precision cancer imaging

Stefan Harmsen *et al.* (Moritz Kircher)

Citation
Sci. Transl. Med. 21 Jan 2015:
Vol. 7, Issue 271, pp. 271ra7

10.1126/scitranslmed.3010633

- A new generation of gold-silica surface-enhanced resonance Raman scattering nanoparticles were created
- These particles can image microscopic and premalignant mouse and human lesions
- The particles are safe in mice and may improve cancer imaging and surgical resection



CREDIT: HARMSEN *ET AL./SCIENCE TRANSLATIONAL MEDICINE*

The margins of most cancer types are not well demarcated because the cancer diffusely infiltrates the surrounding tissues. Cancers also may be multifocal or microscopic satellite lesions, which lead to persistence, local recurrence, and metastatic spread, and are difficult to visualize with currently available imaging technologies. Surface-enhanced resonance Raman scattering (SERRS) nanoparticles, termed SERRS-nanostars, allow for precise visualization of tumor margins, microscopic tumor invasion, and multifocal locoregional tumor spread. The SERRS-nanostars feature a star-shaped gold core and a Raman reporter resonant in the near-infrared spectrum. In five different mouse models of cancer and two models of premalignant lesions, SERRS-nanostars enabled accurate detection of macroscopic malignancy as well as microscopic disease, without the need for a targeting moiety. High sensitivity and broad applicability, in conjunction with the inert gold-silica composition, render SERRS-nanostars a promising imaging agent for more precise cancer imaging.

BIOENGINEERING

Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells

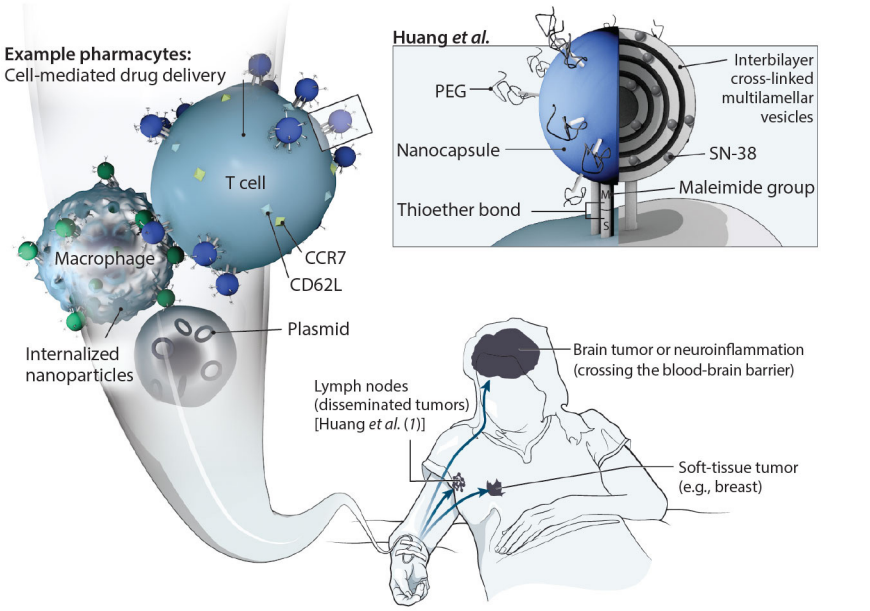
Bonnie Huang *et al.* (Darrell Irvine)

Citation
Sci. Transl. Med. 10 Jun 2015: Vol. 7, Issue 291, pp. 291ra94

10.1126/scitranslmed.aaa5447

See the related Focus by Jeffrey Hubbell, "Prescription for a pharmacyte" (291fs23)

- Lipid-based particles containing the anticancer drug SN-38 were bound to T cells
- These "pharmacytes" were able to transit physiological barriers and deliver chemotherapy to remote cancer sites
- The modified T cells expressed homing ligands and targeted lymphomas hidden in mouse lymph nodes



CREDIT: V. ALTOUNIAN/*SCIENCE TRANSLATIONAL MEDICINE*

Tumor cells disseminate into compartments that are poorly accessible from circulation. Healthy lymphocytes can be programmed to phenocopy the biodistribution of the tumor cells, and therefore deliver drugs to these compartments. Autologous polyclonal T cells were expanded ex vivo under conditions that retained homing receptors mirroring lymphoma cells, and these T cells were functionalized to carry chemotherapeutic (SN-38)–loaded nanocapsules on their surfaces. Nanocapsule-functionalized T cells were resistant to SN-38, but mediated efficient killing of lymphoma cells in vitro. Upon adoptive transfer into mice, the T cells served as active vectors to deliver the chemotherapeutic into tumor-bearing lymphoid organs. Cell-mediated delivery concentrated SN-38 in lymph nodes, reduced tumor burden significantly, and enhanced survival under conditions where free SN-38 and SN-38–loaded nanocapsules alone were ineffective. Tissue-homing lymphocytes can therefore serve as targeting agents to deliver nanomedicine into inaccessible sites, and thus improve the therapeutic index of chemotherapeutic drugs with unfavorable pharmacokinetics.

CANCER

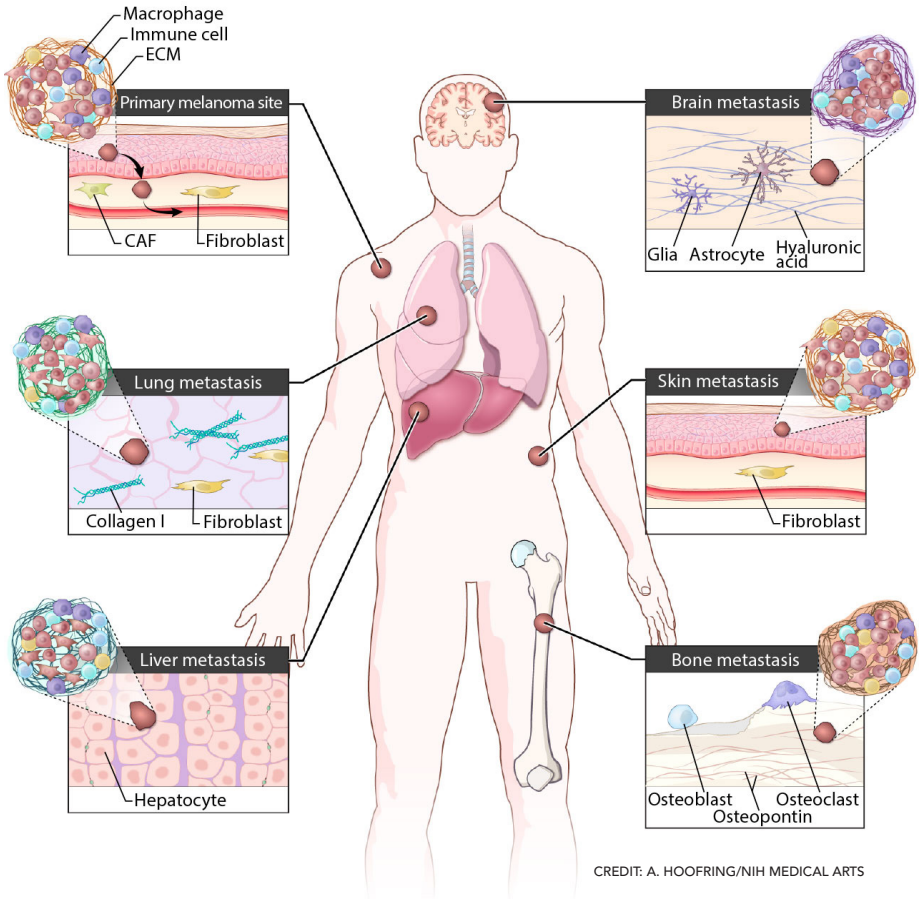
Beyond 3D culture models of cancer

Kandice Tanner and Michael M. Gottesman

Citation
Sci. Transl. Med. 15 Apr 2015:
Vol. 7, Issue 283, pp. 283ps9

10.1126/scitranslmed.3009367

The mechanisms underlying the spatiotemporal evolution of tumor ecosystems present a challenge in evaluating drug efficacy.



Designing in vitro platforms recapitulating diverse in vivo microenvironments. Tumor cells may adopt different morphologies, patterns of ECM secretion, and modes of migration to successfully colonize distal organs. Clinically, cutaneous melanoma shows a broad tissue tropism and ability to metastasize to many organs. This illustration shows the architectural complexity at each of the diverse microenvironments in which both cell type and ECM composition might affect treatment efficacy. CAF, cancer-associated fibroblast.

affymetrix
Biology for a better world

Join the innovators who are creating their own path to get the best results.

Are you a **next-gen scientist?**

A next-gen scientist is someone who follows the science, not the technology trends.
Someone who believes that fast, actionable results are what matter.
That doing what's right for each project is essential.
That data is most valuable when it advances human health and well-being.

If this sounds like you, then you're a next-gen scientist.

Find out what type of next-gen scientist you are at
www.affymetrix.com/nextgenscientist