References

1. Kwon HJ, Lee JW, Chung NG et al. Assessment of serologic immunity to diphtheria-tetanus-pertussis after treatment of Korean pediatric hematology and oncology patients. J Korean Med Sci 2012; (1): 78–83.
2. Nilsson A, De Milito A, Engstrom P et al. Current chemotherapy protocols for childhood acute lymphoblastic leukemia induce loss of humoral immunity to viral vaccination antigens. Pediatrics 2002; 109(6): e91.
3. Ljungman P, Lewensohn-Fuchs I, Hammarstrom V et al. Long-term immunity to measles, mumps, and rubella after allogeneic bone marrow transplantation. Blood 1994; 84(2): 657–663.
4. Ljungman P, Wiklund HM, Duraj V et al. Response to tetanus toxoid immunization after allogeneic bone marrow transplantation. J Infect Dis 1990; 162(2): 496–500.
5. Delacruz W, Terrazzino S, Osswald M et al. Implementing a multidisciplinary approach to enhance compliance with guideline-recommended prechemotherapy pneumococcal vaccination in a military-based medical oncology practice. JOP 2017; 13(11): e966–e971.
6. Poeppl W, Lagler H, Raderer M et al. Influenza vaccination perception and coverage among patients with malignant disease. Vaccine 2015; 33(14): 1682–1687.
7. Locher JL, Rucks AC, Spencer SA et al. Influenza immunization in older adults with and without cancer. J Am Geriatr Soc 2012; 60(11): 2099–2103.
8. Underwood JM, Townsend JS, Stewart SL et al. Surveillance of demographic characteristics and health behaviors among adult cancer survivors–Behavioral Risk Factor Surveillance System, United States, 2009. MMWR Surveill Summ 2012; 61(1): 1–23.
9. Ariza-Heredia EJ, Gulbis AM, Stolar KR et al. Vaccination guidelines after hematopoietic stem cell transplantation: practitioners' knowledge, attitudes, and gap between guidelines and clinical practice. Transpl Infect Dis 2014; 16(6): 878–886.
10. Institute of Medicine Committee to Review Adverse Effects of Vaccines. In Stratton K, Ford A, Rusch E, Clayton EW (eds) Adverse Effects of Vaccines: Evidence and Causality. Washington (DC): National Academies Press (US) 2011. Copyright 2012 by the National Academy of Sciences. All rights reserved.
11. Bitterman R, Eliakim-Raz N, Vinograd I et al. Influenza vaccines in immunosuppressed adults with cancer. Cochrane Database Syst Rev 2018; 2: CD008983.
12. Beck CR, McKenzie BC, Hashim AB, Harris RC. University of Nottingham Influenza and the ImmunoCompromised (UNIIC) Study Group, Nguyen-Van-Tam JS. Influenza vaccination for immunocompromised patients: systematic review and meta-analysis by etiology. J Infect Dis 2012; 206(8): 1250–1259.
13. Chiou WY, Hung SK, Lai CL et al. Effect of 23-valent pneumococcal polysaccharide vaccine inoculated during anti-cancer treatment period in elderly lung cancer patients on community-acquired pneumonia hospitalization: a Nationwide Population-Based Cohort Study. Medicine (Baltimore) 2015; 94(26): e1022.
14. Rieger CT, Liss B, Mellinghoff S et al. Anti-infective vaccination strategies in patients with hematologic malignancies or solid tumors – guideline of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Medical Oncology (DGHO). Ann Oncol 2018; 29(6): 1354–1365.
15. Rubin LG, Levin MJ, Ljungman P et al. 2013 IDSA clinical practice guideline for vaccination of the immunocompromised host. Clin Infect Dis 2014; 58(3): e44–100.
16. National Comprehensive Cancer Network. Prevention and Treatment of Cancer-Related Infections (Version 1.2018) . https://www.nccn.org/professionals/physician_gls/pdf/survivorship.pdf (12 April 2018, date last accessed).
17. National Comprehensive Cancer Network. Survivorship (Version 3.2017). https://www.nccn.org/professionals/physician_gls/pdf/infections.pdf (12 April 2018, date last accessed).
18. Toleman MS, Herbert K, McCarthy N, Church DN. Vaccination of chemotherapy patients—effect of guideline implementation. Support Care Cancer 2016; 24(5): 2317–2321.

doi:10.1093/annonc/mdy154
Published online 24 April 2018

Further evidence to support judicious use of antibiotics in patients with cancer

Cancer immunotherapy centered on blockade of the programmed death receptor (PD1) and ligand (PDL1) has become an essential therapeutic approach for many advanced cancers. A chorus of research reports have focused on tumor-intrinsic factors associated with response to therapy, such as expression of interferon-γ associated genes including PDL1 or tumor mutational burden, as well as mechanisms of resistance such as genomic deletion of class I major histocompatibility complex [1]. Other factors impacting systemic immunity and immunotherapy efficacy are additionally of growing interest including patient germline genetics and environmental or microbiota based factors.

The role of the microbiota, particularly the fecal microbiome, in human disease is increasingly being recognized to play a major etiologic and treatment modification role. In diseases ranging from neurologic and endocrine to autoimmune or cancer a growing number reports are detailing an essential role. The human fecal microbiome is the largest microbiome reservoir in the body and is naturally transferred vertically from mother to fetus at birth. While commonly thought of in terms of bacterial populations, it is important to note that this additionally includes archaea, viruses, fungi and meiobenthos such as protozoa and helminths. This diverse ecosystem is exquisitely sensitive to many factors of daily human life but particularly the intake of medications [2]. A growing literature describing the microbiome as an etiologic agent in driving cancer growth is emerging with multiple reports suggesting an impact on treatment outcomes for chemotherapy [3, 4] as well as immunotherapy [5].

Mouse models and human studies suggest that modulation of the fecal microbiome has major impact on outcomes of cancer immunotherapy both regarding toxicity and efficacy. In hematologic malignancies, reports from patients who have undergone allogenic bone marrow transplant suggest that the diversity of the fecal microbiome at baseline is associated with relapse following treatment while antibiotic use during the transplant course is associated with increased frequency of graft versus host disease and inferior overall survival [6, 7]. Along similar rationale, multiple groups have detailed associations of lower microbial diversity and the presence of certain bacteria in association with efficacy and toxicity from the anti-CTLA4 antibody ipilimumab in melanoma [8, 9]. More recently, a series of studies from patients with advanced melanoma, renal cell carcinoma (RCC) or non-small-cell lung cancer (NSCLC) has suggested associations of distinct bacterial populations with improved efficacy of PD1/L1 blockade [10–12].

Within one of these reports investigating PD1 outcomes, the group from Gustave Roussy in Paris made an initial observation

for a deleterious impact of antibiotic use on outcomes for PD1 based immunotherapy in patients with RCC, NSCLC and urothelial bladder cancer. In the current issue of *Annals of Oncology*, Derosa et al. expand upon this observation reporting on a more robust sample now including patients treated in Europe and the United States [13]. In cohorts of patients including 121 RCC and 239 NSCLC they observed antibiotic use within 30 days of first dose of checkpoint blockade in 13% and 20%, respectively. The outcomes of these patients were inferior to the non-antibiotic treated patients with multivariate analysis suggesting a significantly worse progression-free survival in RCC and overall survival in NSCLC. Interestingly this effect did not extend to antibiotic usage beyond 3 months as there was no effect in the 3–6 month pre-treatment time window. This work was retrospective in nature and results need to be taken with caution. That being said, the results are conceptually striking and consistent with prior basic and translational research.

The implications of this work raise many immediately pressing questions. A short non-exclusive list of such questions might include the following. Are certain antibiotics potentially more immunosuppressive than others? What is the mechanism whereby the microbiome communicates to the tumor microenvironment? Can we supplement the microbiome with a probiotic or perhaps identify circulating factors made by a healthy microbiome to promote antitumor immunity? What about the impact of other antibiotics such as antiviral or antifungal agents? In addition to efficacy, does antibiosis impact on toxicity, either in terms of incidence or level/length of immunosuppression required to manage immune-related adverse events?

Fortunately, and excitingly, several groups have demonstrated the feasibility of microbiome transfer from human patients into murine systems and replicated the clinical phenotype in the mice [10–12]. This approach may facilitate the ability to study some modulation of the microbiome via *in vivo* preclinical models that can then be reverse translated to supplement current treatment paradigms. Conversely an important point to raise may also be the identification of tumor-resident bacteria in pancreas and colon cancers that may limit the efficacy chemotherapy [14, 15] and immunotherapy [16] as well as likely other tumors or treatment modalities [17]. In this setting then depletion of pathogenic bacteria will be a priority in addition to promotion of a healthy commensal environment.

As quickly as cancer immunotherapy has arrived to change standards of care across tumor types, our understanding of the complexity of the tumor microenvironment and perhaps equally important the systemic host response is rapidly changing. The importance of the fecal microbiome in cancer is clearly an emerging area requiring a major focus. Multiple prospective interventional trials are on-going to investigate microbiome modulation in conjunction with standard therapies and it should be emphasized that collection of at least baseline fecal microbiome biomarker samples in clinical trials will be important moving forward. Clinically, it must be emphasized that the cancer community is only at the beginning of our understanding of the microbiome and no evidence based clinical recommendations can yet be given to patients surrounding the use of probiotics. Limiting the use of antibiotics only where necessary should be a priority, however, given the data of Derosa et al., the precarious emergence of drug-resistant pathogens and general medical best practices.

J. J. Luke[1*] & S. K. Pal[2]
[1]University of Chicago, Chicago; [2]City of Hope, USA
(*E-mail: jluke@medicine.bsd.uchicago.edu)

Funding

JJL is supported by Department of Defense Career Development Award (W81XWH-17-1-0265), the National Cancer Institute (P30CA014599-43S), Team Science Award from the Prostate Cancer Foundation (16CHAL12), the Arthur J Schreiner Family Melanoma Research Fund, J. Edward Mahoney Foundation Research Fund and Brush Family Immunotherapy Fund as well as support from Center for Research Informatics of The University of Chicago Biological Science Division and The Institute for Translational Medicine/CTSA (NIH UL1 RR024999).

Disclosure

Consultancies to: 7 Hills, Actym, Amgen, Array, AstraZeneca, BeneVir, Bristol-Myers Squibb, Castle, CheckMate, Compugen, EMD Serono, Gilead, Janssen, Merck, NewLink, Nimbus, Novartis, Palleon, RefleXion, Syndax, Tempest, WntRx with research support to from AbbVie, Array, Boston Biomedical, Bristol-Myers Squibb, Celldex, CheckMate, Corvus, Delcath, Five Prime, Genentech, Immunocore, Incyte, MedImmune, Macrogenics, Novartis, Pharmacyclics, Palleon, Merck, Tesaro, Xencor and travel reimbursement from Amgen, Array, AstraZeneca, BeneVir, Bristol-Myers Squibb, Castle, CheckMate, EMD Serono, Gilead, Janssen, Merck, NewLink, Novartis, RefleXion. JJL is a co-inventor on a patent submitted by the University of Chicago covering use of microbiota to improve cancer immunotherapy outcomes. PS: Consulting: Genentech, Aveo, Eisai, Roche, Pfizer, Novartis, Exelixis, Ipsen, BMS, Astellas; Honoraria: Genentech.

References

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science 2018; 359(6382): 1350–1355.
2. Maier L, Pruteanu M, Kuhn M et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 2018; 555: 623–628.
3. Viaud S, Saccheri F, Mignot G et al. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. Science 2013; 342: 971–976.
4. Iida N, Dzutsev A, Stewart CA et al. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013; 342(6161): 967–970.
5. Sivan A, Corrales L, Hubert N et al. Commensal bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. Science 2015; 350: 1084–1089.
6. Peled JU, Devlin SM, Staffas A et al. Intestinal microbiota and relapse after hematopoietic-cell transplantation. JCO 2017; 35(15): 1650–1659.
7. Shono Y, Docampo MD, Peled JU et al. Increased GVHD-related mortality with broad-spectrum antibiotic use after allogeneic hematopoietic stem cell transplantation in human patients and mice. Sci Transl Med 2016; 8(339): 339ra71.

8. Dubin K, Callahan MK, Ren B et al. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. Nat Commun 2016; 7: 10391.
9. Vetizou M, Pitt JM, Daillere R et al. Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 2015; 350(6264): 1079–1084.
10. Matson V, Fessler J, Bao R et al. The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients. Science 2018; 359(6371): 104–108.
11. Gopalakrishnan V, Spencer CN, Nezi L et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. Science 2018; 359(6371): 97–103.
12. Routy B, Le Chatelier E, Derosa L et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. Science 2018; 359: 91–97.
13. Derosa L, Hellmann MD, Spaziano M et al. Negative association of antibiotics on clinical activity of immune checkpoint inhibitors in patients with advanced renal cell and non-small-cell lung cancer. Ann Oncol 2018; 29(6): 1437–1444.
14. Geller LT, Barzily-Rokni M, Danino T et al. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017; 357: 1156–1160.
15. Guthrie L, Gupta S, Daily J, Kelly L. Human microbiome signatures of differential colorectal cancer drug metabolism. NPJ Biofilms Microbiomes 2017; 3(1): 27.
16. Pushalkar S, Hundeyin M, Daley D et al. The pancreatic cancer microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov 2018; 8(4): 403–416.
17. Garcia-Gonzalez AP, Ritter AD, Shrestha S et al. Bacterial metabolism affects the C. elegans response to cancer chemotherapeutics. Cell 2017; 169: 431–441.e438.

doi:10.1093/annonc/mdy153
Published online 24 April 2018

Methylation in cell-free DNA for early cancer detection

Liquid biopsies and, in particular, analysis of cell-free DNA (cfDNA), have emerged as a promising and potentially transformative non-invasive diagnostic approach in oncology [1, 2]. cfDNA is composed of fragmented DNA released by cells into the circulation, typically as a result of cell death. cfDNA found in the plasma of healthy patients is composed of germline DNA released by normal cells. In cancer patients, some component of the overall cfDNA is composed of DNA released by tumor cells, often termed circulating tumor DNA (ctDNA). The fraction of ctDNA amidst the background of overall cfDNA is highly variable and often low, particularly in patients with early stage cancers [3]. Thus, the detection and analysis of tumor-derived cfDNA poses several challenges and has required the development of specialized technologies with high analytical sensitivity and specificity.

While recent studies have demonstrated the clinical potential of cfDNA for tumor genotyping and blood-based tracking of therapeutic response and resistance [4–7], one of the most transformative potential applications of cfDNA analysis is to detect the presence of cancer in patients without clinical evidence of disease. This potential has been demonstrated most clearly through the detection of residual disease following curative intent cancer surgery [8–11]. By screening post-operative cfDNA for the presence of specific mutations identified in the patient's resected tumor, detection of these tumor-specific mutations using highly sensitive techniques (often capable of detecting mutant alleles present at a frequency of 0.01%–0.1% or less) can accurately identify those patients who will eventually recur.

Similarly, the potential to detect nascent cancers in asymptomatic individuals with a simple screening blood test when they are still curable could revolutionize cancer medicine. Most studies to date utilizing cfDNA for cancer detection have focused on the detection of mutations in cancer-related genes. However, this approach for early cancer detection poses several key challenges. First, cfDNA levels in patients with early-stage cancers are often much lower than with advanced disease [3]. Second, unlike the residual disease setting discussed above, there is no prior knowledge of what specific mutations might be present in an individual patient's tumor. Third, benign lesions may harbor some of the same mutations commonly seen in certain tumors, leading to potential false positives. For example, *BRAF V600* mutations, which are present in nearly 7% of advances cancers, are often observed in benign nevi [12]. Many mutations detectable in cfDNA can also originate from the bone marrow through a process known as clonal hematopoiesis of indeterminate potential (CHIP), which increases exponentially with age [13]. Indeed, evidence of CHIP is observed in 10%–15% of patients over the age of 70 years. Finally, since many cancers share common mutations in genes such as *TP53* and *KRAS*, which are mutated in ∼50% and ∼20% of all cancers, respectively, localizing an early cancer to a specific organ site following the detection of mutations in cfDNA poses a significant challenge.

In this study, Liu et al. evaluate an alternative approach for early cancer detection based on assessing the methylation status of thousands of CpG sites in cfDNA [14]. Indeed, widespread methylation changes are commonly observed across multiple cancer types, with tumors of certain tissue origin displaying specific methylation patterns [15]. Thus, there are several potential advantages of assessing methylation. As mutation detection in cfDNA focuses on changes in a finite number of genes, these techniques are limited not just by analytical sensitivity and specificity, but also by the absolute number of cancer genomes present in a single tube of blood. If no DNA fragment from a specific mutated locus is present in single blood draw, no technique, no matter how perfect, can identify the presence of cancer. However, given the widespread methylation changes present in most cancers, assessing thousands of CpG sites increases the chances that tumor-derived DNA may be detectable in a given blood sample. Another key advantage is that methylation patterns often reflect the epigenetic origin of specific cancers and have been used to unmask the tissue of origin for cancers of unknown primary [16]. Thus, if evidence of cancer is detected, this approach offers the potential to interpolate tumor origin from these data to guide clinical efforts to localize and intervene.

To develop this approach, the authors mined the Cancer Genome Atlas database to identify 10 888 CpG sites frequently found to be hypermethylated in 32 tumor types. CpG sites also methylated in control cfDNA isolated from healthy individuals were excluded, resulting in 9322 individual CpG sites for analysis. In brief, whole-genome amplification was carried out on