Laying the Groundwork for a Revolution

Over the last 100 years, lung cancer has grown from an obscure malignancy to the leading cause of cancer death globally. While public health efforts to reduce tobacco use can impact the rates of smoking-associated cancers, other methods must be brought to bear for the relatively small but significant number of lung cancer patients with no smoking history. The genomics revolution has brought about the promise of targeted therapy for these patients, as the work of past decades set the stage for the discoveries of today. ***Bruce Johnson, M.D.****, former Head of the Lung Cancer Biology Section in the NCI Medicine Branch (now the CCR Medical Oncology Branch) and current Director of the Lowe Center for Thoracic Oncology at Dana-Farber Cancer Institute and Principal Investigator of the NCI-sponsored Specialized Program of Research Excellence (SPORE) in lung cancer at the Dana-Farber/Harvard Cancer Center, offers his thoughts on how scientific foundations laid 20 years ago are now supporting a transformation in lung cancer care.*

It is hard to believe that in the early 1900s, lung cancer was rare enough to be considered a reportable disease. The renowned surgeon Alton Ochsner, one of the first to document the link between tobacco and lung cancer, once remarked that as a student in 1910, he was asked to view an autopsy of a lung cancer patient on the grounds that the disease was so rare he might never have the chance to see another case.[1] Contrast this view with the number of lung cancer cases that we see today: The American Cancer Society estimates that nearly 162,000 people will die of lung cancer this year just in the United States. Lung cancer now claims more people than colon, breast, and prostate cancer combined.[2]

However sobering these numbers may be, there is, of course, cause for hope. The number of people dying from lung cancer is going down. This trend is due to the nature of lung cancer as, primarily, a disease of tobacco use. The epidemic rise of lung cancer in the 20th century can, in large part, be tied to the rise in popularity of smoking in the years during and following World War I.[3] The continued development and deployment of effective tobacco control strategies, starting in the latter half of the century and carrying forward into the present day, promise to have a lasting dampening effect on lung cancer prevalence and mortality.

As the methods for lung cancer prevention have evolved, the methods for lung cancer therapy have similarly advanced. Surgery remains a mainstay of treatment, much as it has been for the last 50 or so years. Radiotherapy has improved, thanks to the development of techniques and technologies that allow the focused application of high doses of radiation directly to a tumor with minimal exposure to surrounding healthy tissues. Chemotherapy has also improved, but it has been applied in an overly broad way. The dominant paradigm has been to treat 100 percent of patients with the same approach to achieve a 20 to 30 percent response rate.

When I started working at the NCI's Medicine Branch as a Clinical Associate, my colleagues and I recognized that characterizing tumor samples genetically would be crucial for the ongoing development of lung cancer therapy. For instance, one of the first things that my mentor, John Minna, M.D. (now at the University of Texas Southwestern Medical Center), and I investigated was the link between *C-MYC* amplification and survival in small-cell lung cancer. But we also recognized that for such developments to come to fruition, we would need to have at our disposal a sizable sampling of tumors large enough to capture infrequent but clinically and biologically important mutations.

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Based on this reasoning, Minna and Adi Gazdar, M.B.B.S. (also at UT Southwestern), set out to systematically generate cell lines from nearly every lung

cancer patient who came to the NIH Clinical Center. Because of our relatively low patient volume, we were fortunate to be able to study our patients very intensively. The patients we saw then numbered in the hundreds annually; in contrast, we see thousands per year just at Dana-Farber. With time and dedication, particularly on the part of the laboratory scientists who actually cultured the tumors that we collected, we were able to create 200 lung cancer cell lines (representing between 20 and 30 percent of patients who crossed the Clinical Center's threshold) while I helped annotate those lines with comprehensive clinical and outcome information for each patient.

At the time that we started these efforts, back in the 1980s, some thought that it was a lot of work for little benefit, that the resources we needed to do this systematic sampling could be better used in other ways. However, these efforts have proved to be more valuable than we suspected at the time. For instance, in 2004, my colleagues at Dana-Farber and I uncovered an association between specific mutations in the epidermal growth factor receptor (EGFR) and the responsiveness and outcomes of patients with non-small cell lung cancer (NSCLC) treated with the EGFR inhibitors gefitinib (Iressa®) and erlotinib (Tarceva®). The first cell line that we found that matched the sensitivity to these two compounds that we saw in patients with this mutation, a cell line called NCI-3255, was one developed as part of this systematic sampling project. This cell line was collected from a woman with an adenocarcinoma who had no history of smoking, a clinical profile that matched the profiles of patients responding to these drugs and who also had the same mutation.

This same cell line also revealed to a trainee and now colleague of mine, Pasi Janne, M.D., Ph.D., one of the mechanisms by which initially sensitive lung tumors can become resistant to EGFR inhibitors, as generally happens within one to two years of treatment with gefitinib or erlotinib. Through the Lung Cancer SPORE program at the Dana-Farber/Harvard Cancer Center, we found that the tumors of some patients treated with these drugs developed a compensating EGFR mutation called T790M. To prove that this new mutation was responsible for this resistance, Janne exposed the NCI-3255 cell line to increasing concentrations of gefitinib for six months. Characterization of the now drug-resistant cell line revealed the same compensatory mutation.

In 2007, a Japanese group announced the discovery of a link between clinical outcomes in a small percentage of NSCLC patients and a genetic translocation called EML4-ALK. As with EGFR mutations, this translocation was discovered more frequently in women with adenocarcinomas who did not smoke; it appears to arise in only about two to three percent of NSCLC tumors. Having found that another of the cell lines we developed at NCI, called NCI-3122, contains this translocation, we have been able to characterize this translocation *in vitro*, develop an *in vivo* model, and begin to study ALK inhibitors as targeted lung cancer treatments.

The revelations we and others generate with these cell lines work both ways. By exposing an additional cell line started by Gazdar and Minna, HCC827, to gefitinib for one year, we discovered that a different genomic alteration, an amplification of the oncogene *MET*, can also give rise to EGFR inhibitor resistance. Going back to archived tumor samples, we have found the same amplification in 20 percent of gefitinib- or erlotinib-sensitive lung cancers that developed resistance.

The list of potentially druggable mutations discovered and characterized using these cell lines continues to grow. And it is doing so at a remarkable pace; the discoveries of the T790M mutation and *MET* amplification noted above happened in the span of two years. As the list grows, a new appreciation of lung cancer's molecular heterogeneity has emerged. Each of these mutations appears only infrequently, at rates ranging between 2 and 10 percent of NSCLC tumors. Because we created so many cell lines with CCR, it is possible to identify at least one cell line for each of these rare mutations, test the cells with different agents, select those agents to which the cells show the greatest sensitivity, and translate them into clinical application.

This heterogeneity in lung cancer tumors and cell lines provides an opportunity to generate an overall model of cancer genomics in translational research. An issue with which all physician-scientists struggle is how to gather enough of a population to study low-frequency events. Lung cancer is a very common malignancy; more than 200,000 people are diagnosed every year, the majority with advanced disease. Thus, by virtue of the sheer number of patients, even infrequent events like EML4-ALK will appear in enough patients to gather large relevant cohorts for clinical trials.

The legacy of commitment to translational research and training at the heart of NCI's intramural program is a driving force behind the national lung cancer research agenda. It should be noted that the leaders of five of the seven funded Lung Cancer SPORE programs in the U.S. are former members of the NCI Medicine Branch, including Minna and me.

And this legacy is fueling what could be a tectonic shift in lung cancer care. With a greater understanding of the frequencies and roles of such mutations in the general population of lung cancer patients, we may be on the verge of flipping the treatment paradigm: By grouping patients based on tumor genomics and treating them with the appropriate targeted therapies, instead of treating 100 percent of patients the same and achieving 20 percent success, we aim to treat 20 percent of patients the same and achieve 100 percent success.

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[1] *Spiro SG and Silvestri GA. One hundred years of lung cancer. Am J Respir Crit Care Med.* 2005;172:523-529.

[2] *American Cancer Society. "What are the key statistics about lung cancer?" http://www.cancer.org/docroot/CRI/content/CRI_2_4_1x_What_Are_the_Key_Statistics_About_Lung_Cancer_15.asp?sitearea=. Accessed* 07/11/08.

[3] *Spiro and Silvestri, ibid.*

(Photo: Courtesy of B. Johnson)

Bruce E. Johnson, M.D.
Professor of Medicine
Harvard Medical School
Principal Investigator
Dana-Farber/Harvard Cancer Center SPORE (Specialized Program of Research Excellence) in Lung Cancer