

Tools of the Trade

Among the best ways to accelerate scientific progress is through the direct sharing of data, tools, and biological materials. Within CCR, most laboratories are contributing directly to this greater scientific good by developing resources for their fellow scientists. Here we profile a few of the many examples of CCR investigators providing cells, techniques, and data sets that are having a worldwide impact on the study of cancer.

Multidrug Resistance

In the 1980s, Michael Gottesman, M.D., now Chief of CCR's Laboratory of Cell Biology (LCB), and Ira Pastan, M.D., now Co-Chief of CCR's Laboratory of Molecular Biology, began a very successful collaboration to understand the increasingly apparent problem of multidrug resistance (MDR) to chemotherapies (see "All in Good Fun," *CCR connections* Vol. 4, No. 2). Their work led to the discovery of the *MDR1* gene, which codes for P-glycoprotein, an ATP-dependent transporter (ABC transporter). This widely distributed membrane protein uses the energy of ATP to actively pump out a broad range of foreign substances from cells, including chemotherapeutic agents. During the course of their research, the team created human cancer cell lines in which drug resistance was enhanced through expression of *MDR1*.

Gottesman's laboratory not only continues to study ABC transporters, it has also developed and become a key source of cell lines, plasmids, and antibodies for research and drug development.

Along with researchers seeking to understand whether ABC transporters act on their drugs of interest, pharmaceutical companies also have a strong interest in these biological resources. "Pharma companies use our cell lines, which are highly characterized, to screen drugs that they are planning to use for cancer treatment," said Gottesman.

(Photo: R. Baer)



Michael Gottesman, M.D., and Carol Cardarelli, B.A.

"In that sense, our cell lines have probably influenced many anticancer drugs in development. The U.S. Food and Drug Administration, in fact, now requires companies to report on whether their drugs are substrates for specific transporters."

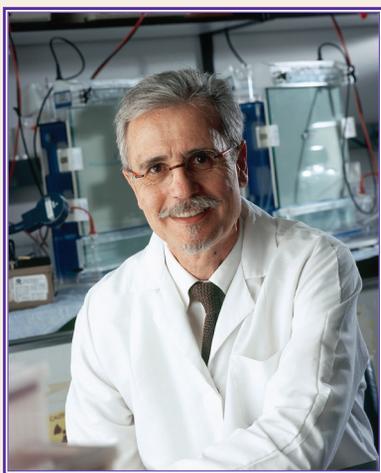
The cell lines are each derived from a single cell and genetically characterized. The most commonly requested cell lines are known as KB-3-1 (drug sensitive parent) and KB-C1, a human cell line derived from the KB-3-1 HeLa line that was initially selected for resistance to high levels of the chemotherapeutic agent colchicine, and then screened for cross-resistance to other unrelated anticancer drugs to characterize their cross-resistance to a broad range of cytotoxic agents. "They are used worldwide as a drug-sensitive control

and MDR-derivative line to study the mechanism of MDR," said Carol Cardarelli, B.A., who has worked with Gottesman since 1983, when the MDR project was just beginning.

Similarly, the KB-V1 cell line, which was the source of the original mRNA used to clone the *MDR1* gene, is routinely used as a drug-resistant positive control for high expression of the P-glycoprotein.

In addition to MDR studies, the research tools are also valuable in the study of drug transport across epithelial barriers. MDCK-pHaMDR is a dog kidney cell line virally infected with the *MDR1* gene. The P-glycoprotein is expressed in a polarized manner on the apical surface of the cells, just as it is in a normal kidney. "One of the major ways drugs are excreted is through

(Photo: R. Bauer)



Yves Pommier, M.D., Ph.D.

the kidney,” said Gottesman. “By studying monolayer cells that form epithelial barriers, like the kidney, pharma companies can study how the drug is handled in the body: its uptake, distribution, and efflux.”

Cardarelli personally handles the MDR-related biological material transfers that are approved by Gottesman and covered by either a NCI Material Transfer Agreement (MTA) or a NIH Office of Technology Transfer (OTT) Licensing Agreement. From 2000–2010, a total of 335 MTAs, were arranged to fill requests, both domestic and foreign, for either cell lines (484), plasmids (173), or antibodies (37) developed in LCB. (One MTA can cover requests for multiple cell lines, plasmids, and/or antibodies.) Over a longer period of time (1997–2014), the OTT issued 55 Licensing Agreements to the private sector. Currently, there are 10 active OTT Licensing Agreements between the NIH and pharmaceutical companies using LCB cell lines for research and development. “We make these resources available because we want to help cancer patients. The more researchers who have access to these materials, the more help there is to develop better anticancer drugs and treatments,” said Cardarelli. “LCB’s cell lines have gone to every continent except Antarctica.”

NCI-60 and CellMiner

Around the same time that Gottesman began creating MDR cell lines, NCI was also developing a new screening tool for anticancer drugs, a panel of 60 human cancer cell lines derived from nine tissues of origin. Now administered by NCI’s Developmental Therapeutics Program (DTP), the NCI-60 represents some of the most thoroughly studied and carefully curated cancer cells. Over the years, laboratories around the world from academia and industry alike have taken advantage of this resource to test their compounds.

“It emerged very quickly that drugs with similar mechanisms of action had similar activity profiles across the 60 cell lines,” explained Yves Pommier, M.D., Ph.D., Chief of CCR’s Developmental Therapeutics Branch. “One cell line would be consistently sensitive to taxol and another would be completely resistant. The responses of the 60 cell lines to a drug define a profile; compounds that have similar targets have similar profiles.”

The DTP created a database to capture the results from these compound screens and Pommier estimates that around one million compounds have been added to it.

Meanwhile, Pommier became interested in the genomic profiles of these cells and how they might relate to drug sensitivities. His laboratory and others began to extensively characterize these cells across several parameters, including gene expression, copy number variation, whole-exome sequencing, miRNA profiles, and karyotype analysis.

“It became obvious to me that if we could bring all this genomic information together with the drug studies, and provide the tools to enable their use, we could produce a wonderful resource for the community,” said Pommier.

His group decided to expand the publicly available CellMiner (<http://discover.nci.nih.gov/cellminer/home.do>), a web-based open access tool, to include both the DTP drug database and their own genomic database. The goal was to create a user-friendly tool to allow anyone to analyze drug responses with respect to a variety of genomic properties.

Paul Liu, M.D., Ph.D., Senior Investigator, National Human Genome Research Institute (NHGRI), for example, has been studying the interaction between transcription factors RUNX1 and CBF- β in certain forms of leukemia. From a high-throughput assay of close to 250,000 compounds, one hit emerged that directly inhibited interaction of these transcription factors. Using the newly expanded CellMiner, the team could show that sensitivity to this compound correlated with the expression of RUNX1 and CBF- β in NCI-60 cell lines.

The power of the database continues to grow. A recent paper in *PLOS ONE* describes newly added comparative genomic hybridization (array CGH) data; and, through a collaboration with Paul Meltzer, M.D., Ph.D., Chief of CCR’s Genetics Branch, methylation and RNA sequencing (RNAseq) data are soon to be added.

Pommier notes that both the Broad Institute in Mass. and the Sanger Institute in the U.K. have more recently adopted similar, larger-scale approaches through the Cancer Cell Line Encyclopedia (CCLE) and the Cancer Genome Project (CGP), respectively. Each encompasses 1,000 cell lines. The majority of the NCI-60 cell lines are included in these databases. “It’s a rich environment for us to do cross comparisons and mine the uniquely vast NCI drug database,” said Pommier.

(Photo: R. Baer)



Stephen Hewitt, M.D., Ph.D.

Tissue Microarrays

The Tissue Array Research Program (TARP) was created in 2000 to distribute tissue microarrays (TMAs)—slides containing up to 500 different formalin-fixed, paraffin embedded tissues—representing the most common human malignancies, which could be used for immunohistochemistry staining to identify the presence of particular proteins of interest. The technology for construction of TMAs had just been developed by NHGRI and its value to cancer research was quickly recognized.

In addition to applying these standard TMAs, TARP now works with NIH investigators with more specialized needs. “We provide complete lifecycle support,” explained Stephen Hewitt, M.D., Ph.D.,

Staff Clinician in CCR’s Laboratory of Pathology. “We assist in identifying a source of the material, construct the tissue microarray, perform the immunohistochemistry, and provide interpretations.” They have also adapted the NCI-60 cell line as an array for immunohistochemistry, which is available through NCI’s Cancer Therapy Evaluation Program (CTEP).

Among his many collaborations, Hewitt has been working with Udayan Guha, M.D., Ph.D., Investigator in CCR’s Thoracic and Gastrointestinal Oncology Branch. Guha uses mass spectrometry to identify potential therapeutic targets for lung cancer in cell lines, focusing on the epidermal growth factor receptor (EGFR) pathway. “With a postdoc in my lab, we obtained a cohort of 300 lung cancers from Japan and were able to confirm with immunohistochemistry that targets he had identified are clinically relevant,” said Hewitt. This effort has now moved forward to support Guha’s rapid autopsy protocol.

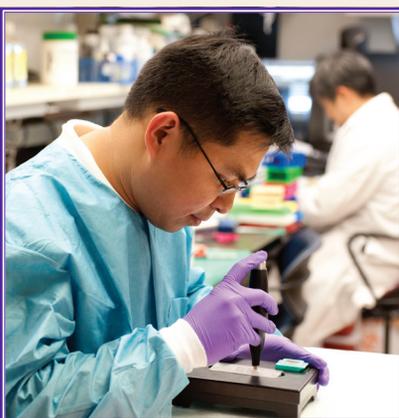
“It’s really not uncommon for studies to take many years to come full circle,” said Hewitt. He has worked with Philip Taylor, M.D., Sc.D., Senior Investigator in NCI’s Division of Cancer Epidemiology and Genetics since 2002, when they

constructed a tissue microarray of squamous cell carcinomas from patients in China. They followed up initial biomarker analysis with a study of patients that were undergoing screening, to identify distinct roles in tumor metastasis. “Recently, we built an array to compare normal tissue with tumors, looking for markers of the earliest stages of disease.”

In addition to tissue microarray work, Hewitt’s group performs antibody validation, troubleshoots biospecimen handling, and develops instrumentation. They filed a patent for a low-cost tissue arrayer in 2003 that was commercialized via the Small Business Innovation Research (SBIR) program. They are in the process of adapting that instrument to construct tissue microarrays out of frozen tissue.

“Target proteins come from all over—mRNA microarrays, mass spectrometry, proteomics, and now genome-wide association studies (GWAS). But at the end of the day, when someone wants to translate them into clinically relevant pathophysiology in large populations and look at outcomes, they basically come down to tissue microarrays and immunohistochemistry,” said Hewitt. “We provide the expertise for that validation.”

(Photo: R. Baer)



Kris Ylaja demonstrates the template arrayer, the latest generation of tissue array instruments.

To learn more about Dr. Gottesman’s research, please visit his CCR website at <http://ccr.cancer.gov/staff/staff.asp?name=mgottesman>.

To learn more about Dr. Hewitt’s research, please visit his CCR website at <http://ccr.cancer.gov/staff/staff.asp?name=shewitt>.

To learn more about Dr. Pommier’s research, please visit his CCR website at <http://ccr.cancer.gov/staff/staff.asp?name=pommier>.