HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

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IN THIS ISSUE

WHAT'S NEW

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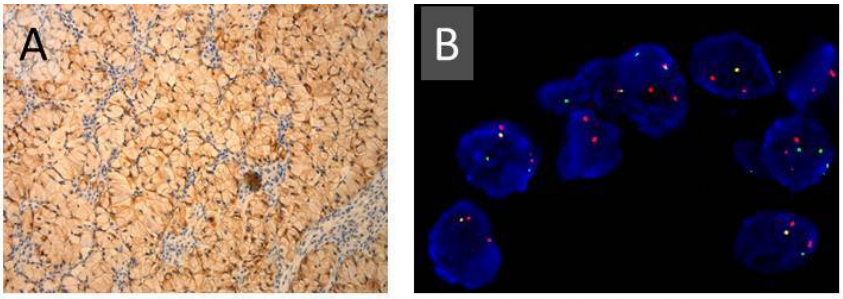
Molecular Testing of Lung Cancers – Rising to the Challenge of Personalized Medicine

November was a month when many moustaches were sported but there seemed to be little awareness that it was also Lung Cancer Awareness Month; a global initiative since 2004. In spite of a lower profile held by lung cancer, recent Canadian cancer statistics [1] indicate that the numbers of new lung cancer cases, about 24 thousand yearly, are comparable to breast, prostate and colorectal cancers. The dramatic difference is in mortality, which is higher in lung than for these three other cancers combined. Equally dramatic however, is the potential response that advanced lung cancer patients can show to an emerging line of highly specific and minimally toxic drugs that target abnormal signaling of tyrosine kinase (TK) receptors [2]. These drugs, TK inhibitors (TKIs), can effectively halt tumor progression and even trigger tumor shrinkage by antagonizing TK receptors such as Epidermal Growth Factor Receptor (EGFR) and Anaplastic Lymphoma Kinase (ALK). However, before a patient may receive such therapy, their tumor must be tested to predict potential sensitivity to the TKI. Pathologists play a central role in this process by acting as gatekeepers in the selection of appropriate tumor samples and in the interpretation of some predictive tests. Between the HRLMP and Bay Area Genetics Laboratory (BAGL), Hamilton is rapidly becoming the regional reference center for this emerging and expanding field of predictive and diagnostic molecular testing.



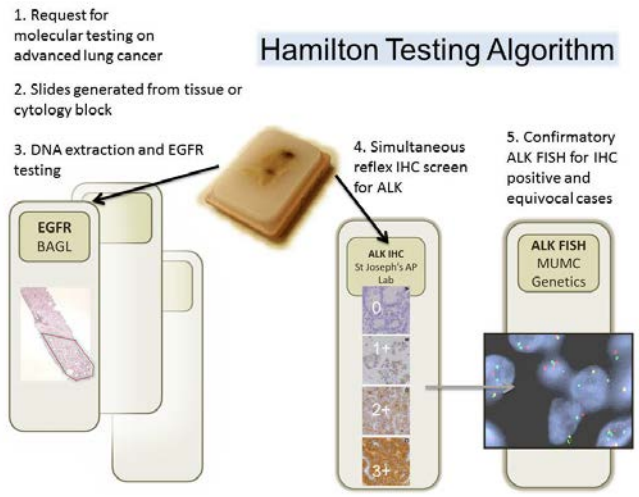
A. ALK Immunohistochemistry on ALK - positive lung adenocarcinoma with "signet ring" morphology associated with ALK gene re-arrangement. 10X original magnification. Photo by J.-C. Cutz. B. FISH showing break apart probe pattern in an ALK-positive case. 100X original magnification. Photo by Diana Munavish Joschko, MUMC cytogenetics Lab.

Given that the Hamilton Niagara Haldimand Brant (HNHB) LHIN surpasses all other local LHINs in the number of new lung cancer cases diagnosed per year, there was enormous pressure to have these predictive tests in place once the drugs became available. Testing for EGFR mutations, which is necessary to preselect patients for the TKI gefitinib, was up and running at BAGL in early 2012. In the year prior, when the drug first came out, samples had to be tested at University Health Network in Toronto. Bringing the test locally was made possible by guaranteed funding for the test, which costs around $400 per patient, through support from the drug manufacturer Astra-Zeneca. Another breakthrough drug then became available in June 2012 called Crizotinib that targets lung cancers harboring a gene rearrangement of ALK [3]. The number of ALK positive patients is very small, 5% or less of those with lung adenocarcinoma, but individual stories of patients responding to the drug have bordered on miraculous. I recently learned of a Toronto patient in an ICU dying of advanced lung cancer who tested ALK positive and upon receiving the first few doses of Crizotinib was out of the ICU and walking about. As a pathologist it is an exciting and even uplifting experience to take part in the identification of ALK positive patients knowing that the therapeutic response can be so immediate and favorable for an otherwise dismal clinical course.

Fortunately, the validation and running of EGFR and ALK testing has gone smoothly, but has not been without some challenges. Molecular testing is done using formalin fixed paraffin embedded tissues or cytology cell blocks. A major issue with these tests is the availability of sufficient tumor tissue or cells for testing ("tissue is the issue"). Many patients with advanced lung cancer have been diagnosed using minimal samples such as core biopsy and cytology specimens and do not have larger resection specimens of their tumors available since surgery is not done for advanced disease. To first reach a diagnosis of metastatic lung cancer it is most often necessary to demonstrate the site of origin, requiring the consumption of more material when performing immunohistochemical stains. What is left in some cases are literally scraps of diagnostic material that must be rationed for EGFR and ALK testing. A local testing algorithm has been developed to efficiently distribute tissue for testing to the various labs in Hamilton and this has yielded sufficient samples in more than 90% of cases.

Collaboration between multiple local laboratories and departments is necessary to provide molecular testing for cancer patients in the region. Requests for molecular testing of lung cancers usually originate from the oncologist who sends a requisition for EGFR testing to BAGL. Samples originating outside HRLMP are first received in the Pathology Department at St Joseph's Hospital where they are assessed for adequacy and suitability along with HRLMP specimens. Tissue sections with different specifications are generated for EGFR and ALK tests at the St Joseph's site histology lab and then sent off to three different laboratories. One set of slides arrives at BAGL to be macrodissected as per the instructions of a pathologist and are subject to DNA extraction and purification. The extracted DNA is then used in a multiplex PCR test designed to detect 28 known EGFR mutations. This represents an extensive and in depth panel that can detect both common and less frequent EGFR mutations and therefore, potentially more patients eligible for TKI therapy. Using a totally different approach, ALK testing first involves screening samples for tumor expression of the ALK protein by immunohistochemistry. Abnormal expression of the ALK receptor can only occur in the presence of a gene rearrangement and a highly sensitive, modified IHC protocol has been developed to detect ALK in lung cancers. ALK IHC is performed using a state of the art Leica autostainer located at the St Joseph's site. Tumor cells or tissues that show any degree of immunoreactivity must then undergo a confirmatory test for the gene rearrangement by fluorescence in situ hybridization (FISH). The cytogenetics lab at MUMC

takes on this step which involves the laborious task of assessing FISH signals in individual tumor cell nuclei for the presence of the gene-rearrangement. As of early December 2012, almost 700 patients have been tested for EGFR and ALK using the algorithm described. Roughly one third of requests are for patients at the Juravinski Cancer Center and the remainder from local cancer centers most notably Grand River, Brantford and Joseph Brant. Local lab data show that detection rates are within the expected ranges published in many studies, around 17% for EGFR mutations and 3.5% for the ALK gene re-arrangement [4, 5].



The Hamilton EGFR/ALK testing algorithm runs smoothly for today's patients but in the long term it is not sustainable given current financial and technical resources. Additionally, it is not compatible with future plans to expand the molecular testing panel to other important predictive markers. The combination of tests for just these two markers approaches $1,000 per patient and is resource and tissue intensive. Many translational pathology and molecular laboratories have moved to more cost-effective multiplex tests and/or high throughput sequencing platforms which assess hundreds of genes or markers. Using these approaches, the cost of testing per gene is reduced by over 90% and they do not require more sample than what current methods consume. The good news is that the most difficult step has been overcome: creating a laboratory infrastructure that can rise to the challenges of personalized medicine.

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Core Laboratory Update

After over a year of planning and preparation, the Core Laboratories went live with their new Abbott Architect chemistry analyzers on November 28th. The Architect family of chemistry analyzers will service approximately 50% of the core laboratory workload which includes testing for over 80 analytes.

The validation process for the new analyzers was thorough and intense. It involved running over 53,000 tests on patient samples on the analyzers at the Hamilton General, Juravinski, McMaster and St. Joseph's Core Laboratories. Core laboratory staff worked tirelessly to complete the validation studies in time for our go-live date.

Special thanks to the key operators who worked night and day to complete the validation studies across all four Architect analyzers on very tight timelines. Our Laboratory Information Services (LIS) team made a tremendous effort to build and validate the required changes in Meditech. Six other project teams also contributed to the successful implementation of these analyzers,

and their work will continue as we move into phase two of the core laboratory project. Kudos to Joan Weppler, Manager, Core Laboratories and SCC; Brenda Rafter-Tadgell, Director of Business Planning; and Kathy Boroski, Project Facilitator, who successfully led the project teams through the analyzer implementation.

A special thank you to all core laboratory staff at all sites who have ensured that our core laboratory service has not been compromised throughout the transition to the new analyzers.

There have been changes to reference intervals and specimen collection requirements for some analytes. Reference intervals are stated on each patient report and specimen collection changes are listed in our Laboratory Test Information Guide which can be found on our website at www.hrlmp.ca.

The implementation of the Architect analyzers completes the first phase of the Core Laboratory project. Phase two of the project includes the implementation of the hub model in which the local core sites (JHCC, MUMC and SJHH) will transport all routine specimens to the hub site at HGH during specified hours. An automated line (pre-analytical track system) will be installed at the General site in 2013. The installation of BioRad Unity and Data Innovations software at all sites will enhance our ability to provide excellent service to our clients. A call centre will be created to better serve our hospital clients providing service for core laboratory inquiries about specimen status, results and add-on testing.

Stay tuned for further updates throughout the winter...

Education News

The Anatomical Pathology, Medical Microbiology and Genetics Training Programs have successfully completed Internal Reviews.

For information and the latest news on our residency training programs follow the link: http://fhs.mcmaster.ca/pathres/news/index.html

Information on the postdoctoral fellowship: http://fhs.mcmaster.ca/pathology/education/postdoctoralfellowshiptraining.html

Chemistry News

With the implementation of the new Chemistry analyzers there have been several changes to note

- Specimen requirements have changed for some assays. Please check specimen labels carefully
- Interval changes have occurred for some assays. Please check reference intervals on reports carefully

There has been a change to **Troponin** testing. The Troponin T test has been replaced by Troponin I testing. The reference intervals for this test are:

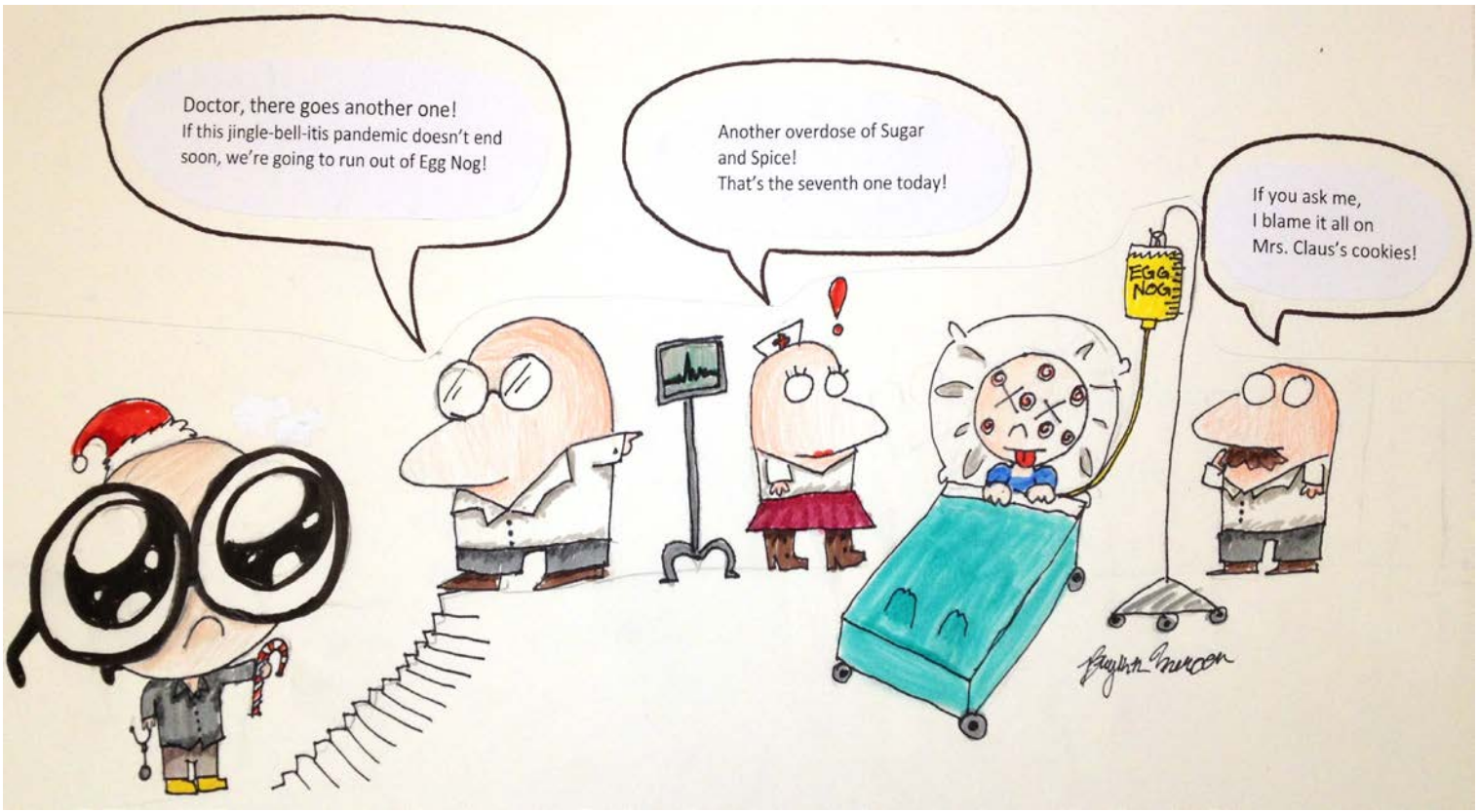
<0.04 µg/L No evidence of myocardial injury
≥0.04 µg/L Evidence of myocardial injury

To evaluate patients with suspected Acute Coronary Syndrome, blood samples for the measurement of troponin I should be drawn on first assessment and repeated 3–6 hours later. Later samples are required if further ischaemic episodes occur, or when the timing of the initial symptoms is unclear.

The following **tumour marker immunoassays** have changed : CEA, CA 125, AFP, CA 15-3, CA 19-9, total PSA (& free PSA), and hCG. From November 28, 2012 until January 19, 2013, each of these immunoassays will be performed on the current analytical platform (Roche Diagnostics) and the new analytical platform. Each sample received during this timeframe will be analysed twice and a dual report will be issued. The new Analytical method and reference interval will be clearly indicated and will follow the current test.

For early detection of pregnancy, continue to order **BHCGQ**. This test is performed on the Abbott Architect. The specimen for this test will be lithium heparin plasma with the detection limit < 2 IU/L; reference interval (female only) <5 IU/L. A total HCG assay has also been introduced for oncology patients. This test can be ordered with the mneumonic **HCGTOTAL**.

Happy Holidays!



Cartoon by Brynn Mercer, age 10

From the HRLMP