LAB Connections

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HAMILTON REGIONAL LABORATORY MEDICINE PROGRAM

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Companion Diagnostics in the era of Next Generation Sequencing

Genetic testing in cancer is done for multiple reasons including refining the diagnosis, providing prognostic information, and assessing the risk for tumor development associated with hereditary cancers. Most recently, cancer genetic testing has also expanded to include personalized genome medicine. The ultimate goal of personalized genome medicine is to tailor therapy choices to target specific genetic alterations identified in each patient's cancer. Development of targeted therapies is thought to hold the most promise for successfully treating cancer, but this targeted approach is dependent on understanding the underlying driver genomic alterations in the tumor cells. A term which is now frequently used to define a diagnostic test capable of identifying patients which would benefit from a specific treatment is "companion diagnostics". This "companion diagnostic" test is used as a companion to a therapeutic drug to determine its applicability to a specific patient.

An important example of success of personalized genome medicine is the seminal work of development of imatinib as a targeted therapy in chronic myeloid leukemia (CML). It took almost 40 years from the initial description of a cancer-related cytogenetic aberration in CML to the clinical application of imatinib, and this highlights how the progress in genetic technologies led to the understanding of the underlying genomic alteration which made possible the development of a highly efficient targeted therapy. In 1960 a small marker chromosome was described in

the leukemic cells of CML patients that was named the Philadelphia chromosome after the city where it was discovered. With the development of banding techniques in the 70s it was shown that the Philadelphia chromosome results from the reciprocal translocation of the long arms of chromosome 9 and 22. In the early 80s the work of several groups allowed identification of a chimeric fusion protein, BCR-ABL1, as the product of this translocation that ultimately promotes CML. ABL1 encodes a tyrosine kinase, and fusion with BCR leads to constitutive activation of the chimeric protein, resulting in uncontrolled proliferation of cancer cells. Further molecular characterization of BCR-ABL1 fusion protein led to the development of imatinib, a drug that inhibits BCR-ABL1 activity by specifically blocking its tyrosine-kinase domain. In 2001, imatinib became the first personalized genomic drug to be approved by the FDA, thus marking a major therapeutic breakthrough and staging the development of novel therapies that target genetic aberrations in other neoplastic disorders [1].

Another successful story of use of personalized genome medicine is the development of targeted therapies in lung cancer. Lung cancer is one of the leading causes of cancer related mortality. On average, 57 Canadians die from lung cancer every day. Non-small cell lung cancer (NSCLC) is the most common form of lung cancer and accounts for 85 to 90% of all lung cancers in Canada. A lot of progress has been made in the past decade in understanding the molecular pathophysiology of NSCLC and developing therapies targeted for specific genetic makeup of lung tumors. For example patients harboring activating mutations in EGFR are sensitive to the tyrosine kinase inhibitors (TKI) gefitinib and afatinib, while patients with ALK and ROS1 fusions are sensitive to a different tyrosine kinase inhibitor, crizotinib. Companion diagnostic is also becoming increasingly important not only at the time of initial diagnosis but also at the time of developing resistance to the first line therapy. One of the mutations tested for EGFR is a p.T790M in exon 20. p.T790M usually develops as a secondary mutation in ~50% of patients with an EGFR sensitizing mutation after about a year of treatment with first-line TKIs [2]. In July 2016 Health Canada has approved a new drug osimertinib as a treatment for patients with locally advanced or metastatic EGFR T790M mutation-positive non-small cell lung cancer. This allows patients to remain on oral treatment longer, delaying the need of chemotherapy. This highlights the importance of genetic testing for treatment decisions and disease monitoring.

Now with the development of new molecular technologies, cancer genetics is accelerating the time from ‘driver mutation discovery’ to ‘clinical proof of-concept’ and the approval of new drugs. While it took almost 40 years from time of the description of Philadelphia-chromosome to the use of targeted therapy for CML, it only took a few years from the discovery of EML4-ALK fusion in lung cancer in 2007 to the development of targeted therapy crizotinib in 2010.

Companion diagnostic tests are becoming increasingly important in multiple types of cancers including melanoma, thyroid, colorectal, brain and many other tumor types. In addition the knowledge of targetable genes in one cancer helps to facilitate the development of similar types of therapies in other tumor types. For example melanoma positive for BRAF codon V600 mutation has been known to be sensitive to BRAF inhibitors, and in June 2017 a new treatment was approved by Health Canada for BRAF V600 positive metastatic non-small cell lung cancer [3].

For many years the HRLMP Cancer Genetics Laboratory has been performing cytogenetics and molecular genetics testing for various hematological malignancies, including CML. HRLMP started offering companion diagnostic tests for solid tumors in 2015, including EGFR testing for lung cancer, KRAS/NRAS testing for colorectal cancer and BRAF testing for melanoma and thyroid cancer. This testing is currently done using real-time allele specific PCR targeting hot-spot mutations associated with positive response or lack of response for specific therapies.

In the fast developing field of personalized genome medicine, however, it has become increasingly important to be able to test multiple mutations in multiple genes from tumor biopsy material which is frequently limited. In this situation Next Generation Sequencing panels are becoming a method of choice in many diagnostic laboratories, allowing multiple genes testing simultaneously and multiplexing samples with different indications in the same batch. An additional advantage of NGS panels is that they allow lower input DNA in comparison to single-analyte methods.

In the HRLMP Cancer Genetics laboratory we have compared data generated by two commercial NGS platforms Illumina TruSight Tumor 15 (TST15) and Life Technologies Ion Ampliseq Cancer Hotspot panel version 2 (CHPV2) using several tumor biopsy samples positive for a spectrum of EGFR, KRAS and BRAF mutations. These panels were compared for the gene regions targeted, DNA input requirements, workflow, automation, capacity to multiplexing, sequence quality, software capabilities and effect on turn-around times. Comparisons of the two kits highlighted distinct strengths and disadvantages for each panel [4]. We have selected Ion Ampliseq Cancer Hotspot panel version for further validation in our laboratory. This panel is focused on point mutation testing of targeted somatic variants in over 50 genes. It will provide opportunities for expansion of the available testing menus and promote participation in a number of ongoing multi-site translational research studies to better understand the role of tumor mutational profiles in personalized genomics and cancer management.

The validation of the panel is approaching completion. We would like to thank **Barry Eng** and **Mackensey Bacon** for their work on validation of the panel. Once validation is complete, updates will be sent out on how the transition to Ampliseq Cancer Hotspot panel will affect the companion diagnostics test ordering.

By:

Dr. Daria Grafodatskaya, Genetics, HRLMP
Dr. Elizabeth McCready, Genetics, HRLMP
Dr. John Waye, Genetics, HRLMP

References:

1. Druker, Blood. 2008, 112(13): 4808-17.
2. Piotrowska & Sequist, 2015, Cancer J. 21: 371–377.
3. Planchard D et al, 2016, Lancet, 17(5):642-50.
4. Grafodatskaya et al, presented at Cancer Genomics Consortium Summer Meeting, Denver, CO, August 8-10, 2016.

News from Administration

RIGHT PATH Team is here again for **2017 BRIGHT RUN**, a very special year as it is the *10th anniversary!*

Together we have raised more than $2 million for breast cancer research, some of this for the funding of HRLMP projects.

The event is being held Saturday, September 9th, 2017 and I hope we have a large group of HRLMP participants, friends and family. Please support BRIGHT RUN 2017 by joining or supporting our RIGHT PATH team!

Secure online donations can be made with your credit card and an electronic tax receipt will be sent to you by e-mail.

Your generosity is greatly appreciated. Together we will make a difference!

For more information about BRIGHT RUN 2017 and to find our *RIGHT PATH team*, visit us at www.brightrun.ca .

On behalf of **Dr. Jennifer Ramsay**, Pathologist, HRLMP

On July 1, 2017, **Dr. Mark Crowther**, became the chair of the Department of Medicine for the Faculty of Health Sciences, McMaster University.

Click on the link below for the full story:
https://fhs.mcmaster.ca/main/news/news_2017/Crowther_chair_of_medicine.html

Dr. Murray Potter has accepted the role of Acting Chair, Pathology and Molecular Medicine, McMaster University.

Click on the link below for the full story
https://fhs.mcmaster.ca/main/news/news_2017/short_term_acting_chairs_named.html

With these changes **Dr. Cheryl Main**, has accepted the position of Acting Department Education Coordinator for the Department of Pathology & Molecular Medicine, McMaster University.

Congratulations to all!

Education News

The **10th Annual HRLMP Rapid Fire Showcase** will be held on Saturday November 25, 2017. Save the date and plan to attend! Further details will be available soon.

The *CSMLS* has recently launched "**LabBuzz**" – a collection of laboratory news and articles delivered to your in-box. Consider subscribing today!

News from Chemistry

Eliminating Urine Beta HCG in the Core Lab - a successful utilization project

Situation

There is a potential cost saving / improved utilization if we discontinue lab Urine Beta HCG. Moreover Plasma Beta HCG is a more sensitive and accurate test.

Currently, all Emergency Rooms have point of care Urine Beta HCG testing available, and the Core Lab has testing available for both urine Beta HCG and Plasma Beta HCG. About 90% of the Core Lab urine Beta HCG tests come from the ER. Therefore, the Core Lab often receives requests for Urine Beta HCG testing even though it has already been performed in the ER (duplicate testing). If lab Urine Beta HCG can be discontinued, it is estimated that a potential 30,000$-40,000$/year can be saved.

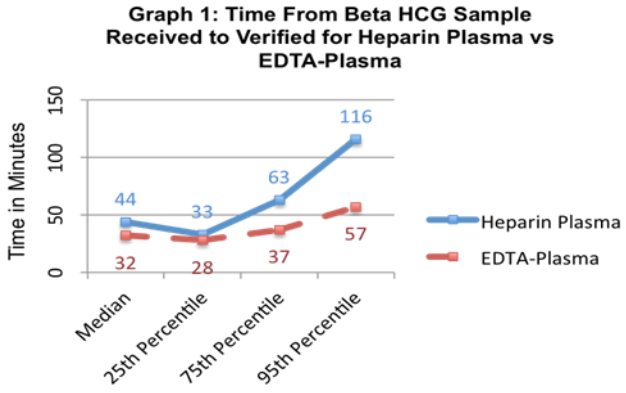
Background

About 2-3 years ago, there had been a recommendation to discontinue lab-based urine Beta HCG, however, the plasma Beta HCG's turn-around time (TAT) was not optimal (~ 2 hours) for patient care. Additionally, some patients require urinalysis and it makes sense to send both the urinalysis and Beta HCG requests to the lab at the same time.

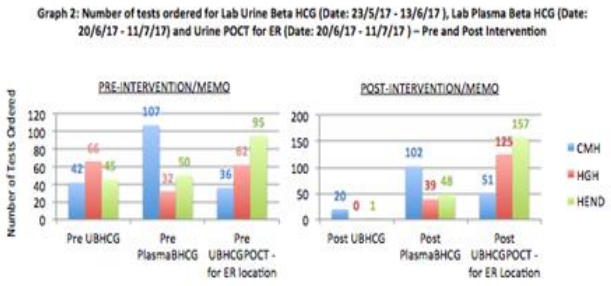
Since this initial recommendation, there has been the introduction of a new sample type for plasma Beta HCG (EDTA Plasma). It has been demonstrated to provide the same results as the previous sample type (heparin plasma), in a significantly shorter TAT (<1 hour).

Analysis

The first analysis compares the turn around time for heparin plasma (green-top tube) HCG sample type testing and EDTA plasma (purple-top tube) HCG sample type testing (Graph 1). This data was pulled at JHCC for 1 year (01/05/16 to 30/04/17). Here we can see that if we switch sample types of Beta HCG from Heparin Plasma to EDTA Plasma, we can decrease the TAT of plasma Beta HCG testing from ~2 hours to <1 hour in 95% of cases.



Next, an intervention was implemented whereby a memo was circulated to all physicians and emergency rooms informing them that the Core Lab will be discontinuing urine Beta HCG testing. The results below (Graph 2) were taken before the memo was sent out and one month later. The results showed that the total number of Core Lab Plasma Beta HCG testing stayed the same, but Urine Beta HCG testing decreased dramatically from 153 to 21. Interestingly, the number of point of care testing for Urine Beta HCG in emergency rooms went up from 193 to 333. This is possibly due to only a month of analysis. Regardless, further work is needed to convince emergency room clinicians that the plasma Beta HCG testing is a superior test.



Recommendation

It is recommended that Core Lab urine Beta HCG testing be discontinued, which will save about 30,000$-40,000$/year. This will eliminate duplicate Urine Beta-HCG testing, as well as promote more accurate testing via high-sensitive plasma Beta HCG testing. The initial pushback to this discontinuation was that the plasma Beta HCG turnaround time was

too long for patient care. It has now been proven that the EDTA plasma HCG sample type can be turned around from 'received to verified' in less than 1 hour in 95% of cases with the same results as previous plasma Beta HCG testing (Heparin Plasma).

As noted in the analysis, further education and a longer data collection period is required to reduce Urine Beta HCG point of care testing in the emergency rooms.

For the future, we would like to see the Urine Beta HCG test eliminated completely from not only the Core lab but also the Emergency Departments. This will increase the sensitivity for testing Beta HCG by solely utilizing plasma Beta HCG testing and will reduce false negative results via urine.

By:
Y. Chetty, T. Carrier, L. Clark, P. Kavsak
Clinical Chemistry and Immunology, HRLMP

Dr. Peter Kavsak, a HRLMP Biochemist, was featured recently in a trade magazine providing details on high-sensitivity cardiac troponin.

The article titled ***High-Sensitivity Troponin I Assays: A breakthrough in AMI Diagnosis*** was published in the August 2017 edition of Heartbeat (siemens.com/cardiology).

Hematology News

Dr. John Eikelboom (Associate Professor, Hematology & Thromboembolism) was announced as the inaugural ***Jack Hirsh/PHRI Chair in Thrombosis and Atherosclerosis Research*.**

Dr. Mark Crowther (Professor, Hematology & Thromboembolism) was awarded a ***BACH Investigator Recognition Award*** at the *XXVI ISTH Congress* in Berlin, Germany in July 2017.

Dr. Sam Schulman (Professor, Hematology & Thromboembolism) was presented with the ***Harold R. Roberts Medal for meritorious service*** at the *XXVI ISTH Congress* in Berlin, Germany in July 2017.

Dr. Donald Arnold is helping to establish the ***Platelet Disorder Support Association*** in Canada, a non-profit patient advocacy group active in the United States.

Click on the link below for the full story (approximately half way down the page):
https://www.thespec.com/news-story/7271893-innovation-notebook/

Dr. Alfonso Iorio is a co-investigator on an international collaboration for setting guidelines on the use of gene therapy in the treatment of Hemophilia.

Click on the link below for the full story:
https://hemophilianewstoday.com/2017/06/21/hemophilia-project-aims-to-set-effectiveness-guidelines-for-gene-therapies/

Registration is now open for the ***13th Annual McMaster Update in Thromboembolism & Hemostasis*** to be held at the Hamilton Convention Centre on Friday October 27, 2017.

Click on the link below for the brochure and registration details:
https://fhs.mcmaster.ca/conted/documents/Brochure_Thrombo_2017_web.pdf

Microbiology News

Organisms growing on the biplate.

The HRLMP **Microbiology Laboratory** is the first laboratory to ever use the chromogenic media biplate (shown above) for MRSA and VRE on the automated WASPLab system.

Microbiology processes over 100,000 MRSA and VRE specimens each year and the implementation of the biplate has decreased the processing time by half and doubled the incubator plate capacity.

Mark Gaskin
Technical Specialist
Microbiology

Special thanks to the HRLMP Biocontainment (aka Ebola) Team

The HRLMP Biocontainment team was disbanded on August 1, 2017, having selflessly served on constant call since the unprecedented Ebola outbreak in 2015. The team was formed to ensure safe and appropriate laboratory specimen collection and transport in patients with suspected hemorrhagic fevers. A special thanks to the team members: **Duane Boychuk, Deb Johnson, Cathie McCallum, Mark Gaskin, Andrea Tjahja, Candy Rutherford, Spencer Brown, Teresa DiFrancesco, John Korver and Dr. Cheryl Main**. No more Ebola BlackBerry calls!

While the team will no longer be on call, members will continue to maintain biosafety skills and will be available as needed to respond to future emergencies.

News from Pathology

Welcome to **Dr. Jian Qiang Lu**, a Neuropathologist, who joined the HRLMP July 4, 2017. Dr. Lu has replaced **Dr Boleslaw Lach** who is now retired. We wish Dr. Lach a very happy retirement!