The experience of the WUSM Genomics Tumor Board and practical issues of integrating comprehensive sequencing platforms into clinical care of cancer patients

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I have no financial disclosures.
GTB0: Familial MDS Project

Approach: Identify germline variants in the two affected family members that are not present in two unaffected family members.

GTB0: Familial MDS

- Sequenced two affected individuals and 2 controls. Identified 3 candidate variants (CBL, CBFA2T3, and EHMT2). Will perform Sanger sequencing on 2 other controls and 1 affected family member (multiple myeloma). Extension set of 12 families with familial MDS of unknown etiology available for screening.
GTB0: Familial MDS Project

- +EHTM2 heterozygous missense germline variant
- NO EHTM2 heterozygous missense germline variant

GTB1: Metastatic SCC of Lung in Setting of Juvenile Respiratory Papillomatosis (JRRP)

- 19 year old male, never-smoker with cerebral palsy, asthma and JRRP with lung involvement
  - s/p laryngoscopies, bronchoscopies and debridements of papillomas every 6-8 weeks since age of 6 months
  - Previously treated with gardasil, interferon. Cidofovir started at age 9
  - Complicated by chronic recurrent respiratory infections
- RLL biopsy with squamous papilloma with high grade dysplasia -> focal invasive carcinoma
Description of samples

Trachea
- squamous papilloma resection
- Squamous papilloma with moderate dysplasia

Larynx
- papilloma resection: HPV 6 or 11

Supraclavicular node SCC excisional biopsy

RLL Lung
- squamous papilloma with high grade dysplasia
- SCC (FNA and bx):

Gastrohepatic lymph node SCC FNA

RLL Lung
- squamous papilloma with high grade dysplasia
- SCC (FNA and bx)
GTB1: Metastatic SCC of Lung in Setting of Juvenile Respiratory Papillomatosis

• The pooled exome capture had a high duplication rate secondary to the low input of DNA from several of the samples. We did detect the presence of HPV 11 in all samples sequenced (no evidence of integration however) using IDT probes spiked into the exome reagent. We will repeat exome sequencing of the metastatic sample and germline sample (blood).

GTB2: Sinonasal Melanoma Case Presentation

• 64 year old woman presented with a 2 month history of nasal congestion. Examination revealed a polyp obstructing the nasal passage and a prednisone taper with antihistamines was prescribed with no improvement. CT confirmed a 5.4 x 1.8 x 3.8 cm mass in the right nasal passage. She developed epistaxis.

10/7/2013 CT: Coronal View

10/7/2013 CT: Axial View
GTB2: Sinonasal Melanoma

- Exome and RNA sequencing of tumor/ paired germline (blood) and analysis are complete. No "actionable" targets identified.
- There was no TERT promoter mutation identified (and had excellent coverage based on IDT spike-in probes)
- Identified 6 potential neo-antigens (2 within NRAS) and are now vaccinating the patient (who had progressive, metastatic disease) with the dendritic cell vaccine as described by Carreno et al.

GTB3: Concurrent Germ Cell Tumor (GCT) and M7 AML

- 33 year old man who initially presented with a 1 month history of generalized weakness, 40 lb weight loss, and dyspnea on exertion
- Noted to have petechial rash
- Initial CBC:
  - WBC: 9.2
  - Hb: 13.1
  - Plts: 5
  - Normal differential
CT Scan Reveals Mediastinal Mass

Pathology

- BmBx: 70% cellularity with a population of large mononuclear cells with irregular contours.
  - Subset of these cells expressed CD61 and weak CD117
- Hemodilute aspirate with 15% large blasts
  - Small percentage of these expressed CD41, CD61, and weak CD117
- Final diagnosis: moderately fibrotic marrow with findings suggestive of **acute megakaryoblastic leukemia (AML M7)**
Clinical Course

- Induction chemotherapy with 7+3
- Day +15 bone marrow biopsy with a chemo-ablated marrow and no evidence of leukemia
- Underwent 2 biopsies of mediastinal mass with pathology showing necrotic tumor and “adenocarcinoma”
  - Evaluation limited by lack of viable tumor tissue
- Treated with one cycle of consolidation with HiDAC
- Given increasing tumor markers treated with 2 cycles of etoposide, ifosfamide, cisplatin (VIP) for germ cell tumor

GTB3: Concurrent Germ Cell Tumor (GCT) and M7 AML

1. Performed WGA on 2 ng DNA isolated from laser capture microdissection of the GCT sample (4 separate WGA reactions were performed—which was pooled prior to capture).
2. We flow-sorted the AML sample but were only able to isolate 50 ng of DNA. We then:
   1. Three PicoPlex WGA reactions (2ng each) and pool DNA
   2. Two-indexed WGS Libraries (300-400 bp shearing)
      a) WGA DNA 1µg pooled
      b) 40ng GTB3 genomic DNA
   3. One Exome capture
      a) Pool library 2a and 2b
      b) NimbleGen V3 exome capture
3. We used skin DNA as the “germline” comparator.
Shared somatic mutations identified in TP53 and PTEN

A. TP53 c.7578213A>del
    (p.R213fs)

B. PTEN c.89692922T>C
    (p.C136R)

C. | TP53 | PTEN |
   |     |      |
   | GCT | 89%  | 100% |
   | AML M7 | 85%  | 98%  |
   | Skin  | 4%   | 4%   |

Copy number analysis of the unamplified AML M7 sample demonstrated amplification of chromosome 12p.
GTB4: Patient with Metastatic Breast Cancer and History of Multiple Other Neoplasms

57 year old female who presented to medical oncology consultation for LEFT breast cancer.

- Screening mammogram demonstrated a lesion in left breast.
- Biopsy LEFT breast confirms invasive ductal carcinoma of the breast. No palpable mass at the time.
- Left breast lumpectomy performed.

Review of Systems:

- Lower left back pain "when she lifted something heavy."
- Shortness of breath on walking for a couple of weeks.
- Lost 10 pounds in 6 months.

PAST MEDICAL HISTORY/PAST SURGICAL HISTORY:

- Hypothyroidism.
- Osteoporosis.
- Fistula repair after colon surgery in 2005, from ureter to colon, this is resolved.
- Ureter repair early 2006.
- Mitral valve prolapse.
- High blood pressure.
- Asthma.
- Chronic diarrhea, for years, unknown etiology, controlled on cholestyramine.
Past Oncologic History:

- Left nephrectomy for Wilms' tumor at 6 years old, received chemo and radiation then; no records available.
- Thyroid cancer at 37 years old, s/p thyroidectomy and radioactive iodine.
- Renal cell cancer at age 42, s/p partial right nephrectomy (only surgical treatment).
- Hyperparathyroidism/parathyroid adenoma s/p 2 gland parathyroidectomy.
- Large colon polyp-adenoma s/p open laparotomy due to size.
- Low grade meningioma discovered on brain imaging during breast cancer work-up.

SOCIAL HISTORY:
Alcohol: Occasional.
Smoking: Never.

FAMILY HISTORY:
Maternal grandmother breast cancer in 90s.
Paternal aunt breast cancer in 70s.
Father had lung cancer and prostate cancer.
Paternal grandfather bladder cancer.
Left breast lumpectomy:

1.0 cm primary excised.

pT1b pN0 = Stage IA

- On pre-op exam by anesthesia, she had decreased air entry over the left lung field what prompted a CXR (pre-op) and then a CT chest (post-op).

CT Chest/Abdomen/Pelvis:

1. Multiple pleural soft tissue nodules associated with a moderate to large left pleural effusion compatible with pleural metastases and malignant pleural effusions in this patient with breast cancer.

2. Multiple indeterminate bilateral pulmonary nodules.

3. Indeterminate left adrenal nodule. Two small hypoattenuating foci in the liver most likely represent cysts, but are indeterminate.
Biopsy revealed METASTATIC CARCINOMA CONSISTENT WITH BREAST PRIMARY to the pleura, so stage IV at diagnosis.

GTB4: Patient with Metastatic Breast Cancer and History of Multiple Other Neoplasms

Patient was seen by the genetic counseling service.

- Genetic testing was performed through Ambry Genetics Laboratory and included analysis of 28 genes. These genes include: APC, ATM, BARD1, BMRPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, SMAD4, STK11, and the TP53 gene.

- Her testing was NEGATIVE with no mutation identified.

- The laboratory did identify a likely benign variant in the MUTYH gene. Two pathogenic mutations in both copies of the MUTYH gene are necessary for MYH-associated polyposis syndrome which she does not have.
GTB4: Patient with Metastatic Breast Cancer and History of Multiple Other Neoplasms

We performed enhanced exome sequencing (V3 + AML RMG) on breast cancer sample (from partial mastectomy) and peripheral blood (as germline comparator). No good candidates were identified so germline WGS performed with analysis now underway.

GTB5: TCGA AML30 s/p 65 cycles of Decitabine

• Performed Whole Genome Sequencing at approximately 60x on bulk bone marrow sample obtained from a patient with AML after 65 cycles of the hypomethylating agent, decitabine.

• Analysis is now underway.
GTB6: Two Patients with CML (Ph+) that Later Developed Ph- AML

• A enhanced exome trio: CML (DNA isolated from block from initial diagnostic bone marrow biopsy), AML (DNA isolation now underway from block of initial diagnostic bone marrow biopsy: 11mm core, >90% cellular with about 40% blasts) and have skin DNA as normal comparator.

• Initial analysis shows no evidence of a shared founding clone between the AML and CML samples in either case.

Analysis question: Are the Ph- AMLs clonally related to the prior CML?
GTB7: A Patient with Primary Myelofibrosis (JAK2 negative) Treated with Ruxolitinib and then the Anti-LOXL2 Monoclonal Ab who Developed Severe Systemic Mastocytosis (SSM)

- 7 exomes:
  1. Normal skin
  2. Flow-sorted CD34+ cells from SSM bone marrow biopsy
  3. Flow-sorted myeloid cells from SSM bone marrow biopsy
  4. Flow-sorted mast cells from SSM bone marrow biopsy
  5. Bulk bone marrow cells from SSM bone marrow biopsy
  6. Bulk bone marrow cells from bone marrow biopsy while on treatment with anti-LOXL2 Ab (just preceding diagnosis of SSM)
  7. Bulk bone marrow cells from bone marrow biopsy while on treatment with ruxolitinib
Other GTB Cases

- GTB8: Metastatic Papillary Thyroid Cancer; 4 exomes, V3 + AML RMG; 1 blood sample (as germline), primary thyroid tumor, metastatic lymph node sample, and metastatic lung sample.

- GTB9: Maxillary Squamous Cell Carcinoma (SCC); 4 exomes, V3 + AML RMG; 1 blood sample (as germline), primary SCC sample, passage 2 (P2) conditionally reprogrammed cell sample (CRCs) and passage 10 (P10) CRCs. Also will perform standard RNA-Seq on primary SCC only, with Tru-Seq Stranded Total RNA kit. Maybe, the RNA-Seq run can be pooled with GTB1 and GTB7?

- GTB10: Familial Papillary Thyroid Cancer; 4 exomes, V3 + AML RMG; it is 2 patients (one blood as germline and one primary tumor sample from each).

- GTB11: 2 Patients with Myelodysplastic Syndrome and Pulmonary Fibrosis; 4 exomes, V3 + AML RMG, skin (germline) and MDS bone marrow samples.

- GTB12: A Case of Congenital Aplastic Anemia; 1 or 2 exomes, V3 + AML RMG, skin (germline) +/- flow-sorted blood sample.

Comprehensive and Integrated Sequence Analysis Methods are Not Trivial

Knowledgebases
- Germline analysis
- Tumor analysis
- Integration
- Interpretation

Reference inputs
- reference genome
- gene annotations
- variant databases

Data inputs
- tumor WGS
- normal WGS
- tumor exomes
- normal exomes
- tumor RNA-seq
- normal RNA-seq

Germline analysis
- ref align
- ref align
- ref align
- ref align
- RNA-seq
- RNA-seq

Tumor analysis
- somatic variation
- somatic variation
- differential expression

Pipelines (models)

Integration

Interpretation

Build outputs

Putative Driver Events

GRCh37
- dbSNP
- Ensembl
- COSMIC

Raw data
- Fastq/bam

BWA
- Varscan
- Streika
- Breakdancer
- Chimerascan
- Tuxedo
- Htsq

DGIdb
- IVA/IPA
- PubMed

Obi Griffith and Malachi Griffith <ogriffi@genome.wustl.edu and mgriffit@genome.wustl.edu>
Interpretation of the Clinical Relevance of Genomic Events Represents a Serious Bottleneck

Summary

- Advances in genomics are making it possible to study cancer samples in a cost and time efficient manner, which will only improve.

- Identifying profiles and targets in patients will soon make it possible to personalize treatment for cancer patients.

- Considering disease complexity, other factors will be still be important such as environment, lifestyle and personal behaviors.

- Patients’ rights will continue to be large part of the conversation as genomics becomes more accessible.
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Legal/Ethics Issues Are Also Not Trivial: GINA (Genetic Information Nondiscrimination Act)

- Enacted in 2008
- Protections regarding insurance and employment
- Insurance - pre-existing condition information cannot be based only on genetic information, premiums/contributions cannot increase, genetic testing cannot be required
- Employment - firing and refusal to hire cannot be based on genetic information, cannot purchase, request or require information, cannot discriminate in salary/wages, future opportunities, terms/conditions
- Some states are taking the GINA a step further
- Example: Massachusetts Genetic Bill of Rights - genetic information and material considered personal property: insurers of long-term care, disability, life and auto cannot deny coverage or change rates; health centers cannot deny services; and data cannot be used to determine credit worthiness