Affordable NGS to support therapeutic decision-making

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- Nothing to disclose
Overview

- Genomics-to-Therapy, the conundrum
- Availability of NGS tests for cancer patients
- Affordability and Clinical Utility/Benefit of NGS tests
- Immunogenomics
Impact of Cancer Genomics Discovery
Identifying therapeutic targets
By directed PCR and capillary sequencing, we determined that ~80% of Iressa responders have EGFR mutations in the tyrosine kinase domain.

*W. Pao et al.*, *PNAS* 2004
Lung Adenocarcinoma: Discovery to Translation

"NGS-based analysis"

- **EGFR mutation**
  - Iressa, Tarceva, Erlotinib

- **EGFR & KRAS mutations**
  - Surgery, radiation, chemotherapy

- **ALK fusion**
  - Crizotinib, Ceritinib

‘NSCLC Heterogeneity’

- Adenocarcinoma (70%)
- Large Cell (10%)
- Squamous Cell (20%)
- Unknown (42%)
- KRAS (30%)
- EML4-ALK (5%)
- BRAF (2%)
- PIK3CA (1%)
- MEK (1%)
- HER2 (2%)
- FGFR4 (2%)
The Genomic Landscape of Cutaneous Melanomas

- Four subtypes identified based on the most prevalent significantly mutated genes
- Triple WT subtype enriched for KIT alterations of all types
- Outcome did not correlate with genomic classification but did indicate targeted therapeutic approaches
- An RNA-based signature in a subset of samples enriched for immune gene expression were associated with improved patient survival

Akbani, R. et al., Cell 2015
Impact of Large-Scale Cancer Genomics Discovery

• Cancer genes are shared across tissue sites
• Cancer genes are altered in many ways
• Specific cellular pathways are perturbed by the combined somatic and germline alterations

Nature Genetics 45: 1113-1120 (2013)
Clinical Availability of NGS Cancer Assays
Availability of NGS Tests: US

- Most academic cancer centers have either a commercial or “local” NGS panel of genes to offer cancer patients.
- US-wide cancer care specialty centers also offer their own version of an NGS gene panel (Caris, Cancer Centers of America).
- Commercial testing is available to anyone who wishes to pay out-of-pocket, with a physician referral.
  - These assays range from NGS panels to exomes to WGS.
- The extreme examples are wealthy individuals who recruit their own molecular tumor boards.
- Increasingly utilized are the germline or constitutional NGS assays for susceptibility to cancer.
Issues Impacting the Availability of NGS Assays

- Institutional commitment to molecular oncology/diagnostics
- Turn-around-time for assay relative to clinical need
- Tissue-site specific number/likelihood of targeted therapy being utilized or clinical trial availability
- Patient-specific disease course (primary vs. metastatic)
- Hotspot versus whole gene assays (VUS)
- Availability/amount of tissues for testing
- Physician-specific “comfort level” with genomics
The Challenge: Data Interpretation

The fact that cancer genes and mutations are shared across tissue sites means we can harness the unbiased nature of NGS assays to tell us the cancer-relevant mutations present in individual patients.

These assays typically take the form of panel tests that survey known cancer genes as discerned by large-scale cancer discoveries.

Our current challenges include the interpretation of a given mutation in a known cancer gene and predicting its response to available therapies.
• Information on the clinical impact of many cancer variants is scattered throughout the published literature
  • Making collecting that information both time and labor-intensive
  • Existing resources do not facilitate computational access
• CIViC acts as a centralized forum for curation, interpretation and debate about variants and their functional/targetable/prognostic or diagnostic impact
• CIViC currently holds 1038 evidence statements covering:
  • 454 variants
  • 209 genes
  • 121 cancer (sub)types
  • 815 published sources

www.civicdb.org
Our crowdsourcing interfaces permit external contributions and open dialogue regarding evidence statements.
DGIdb: Drug Gene Interaction database

Submit your manuscript today at molecularcasestudies.org
Clinical Aspects of NGS Cancer Assays
Affordability, Utility and Benefit
Affordability and Related Issues

- At present, CMS (Medicare) will reimburse as much as $600US for NGS tests of up to 50 genes.
- Several large US health insurance payors have now followed suit, in the metastatic setting.
- Any NGS assay that returns a positive result for a known drug target in an FDA approved tissue site may be reimbursed up to the single gene reimbursement level.
- Many academic cancer centers have taken on the cost of testing using philanthropic funds and requesting reimbursement when possible for single genes.
- There remains a reluctance by the FDA to approve NGS tests as companion diagnostics to provide a therapeutic indication for any cancer drug.
If Available and Affordable, then what??

- MD Anderson study of 2,000 pts with advanced cancer and NGS hotspot testing (48-50 genes)
- 789/2000 (39%) had at least one mutation in a potentially actionable gene; 145 (7.3%) had 2 or more such mutations
- 83/789 (11%) with actionable mutations enrolled in a genotype-matched clinical trial after NGS testing (only 4% overall)
- Frequency of actionable mutations varies widely across tissue sites, open clinical trials also were limiting
- Challenges include getting patients to return after testing, desire for treatment closer to home or decline in performance status
- These numbers reflect similar studies such as SAFIR01, LCMC and SHIVA

F. Meric-Bernstam et al., JCO 2015
Argued that the combination of tissue site biology and the genotype must be taken into consideration when predicting response to targeted therapy.

Called for a more precise definition of “actionable”.

Suggested that using gene expression profiling might improve prediction capabilities for both chemo- and targeted therapies.
Clinical Utility of NGS Cancer Assays

- Enhanced accrual to phase 1 clinical trials, especially “basket” trials
- Improved ability to match patient to drug, often better than FDA approved companion diagnostic
- More efficient use of small tissue biopsies, can inform liquid biopsy-based monitoring and heterogeneity estimates
- VUS confound diagnostic potential, off-label indications problematic, tumor-only assays
- New evidence for larger gene panels in MMR-D diagnoses, may indicate potential to respond to checkpoint blockade therapy
Clinical Utility: MSK-IMPACT and MMR-D Detection

- Compared 224 CRC patients with MSK IMPACT panel NGS test data with their MMR IHC status
- 196 (87%) were MMR-P, 28 (13%) were MMR-D (12 with Lynch Syndrome, 6 sporadic CRC, 10 presumed but not diagnosed LS)
- 100% of MMR-P patients had fewer than 20 mutations on the IMPACT panel
- Of 31 tumors with more than 20 mutations, 28 were MMR-D and 3 were polE mutated (>150 mutations each)
- Argued that an NGS gene panel test could accurately diagnose MMR-D status, clarify LS diagnoses and replace IHC assays
DNA Repair Defects and Checkpoint Blockade Response Potential

B Radiographic Response

- Mismatch repair–proficient colorectal cancer
- Mismatch repair–deficient colorectal cancer
- Mismatch repair–deficient noncolorectal cancer

Change from Baseline in the Sum of Longest Diameters (%)

- 20% increase (progressive disease)
- 30% decrease (partial response)

Dung et al., NEJM 2015
Immunogenomics
Identifying Neoantigens
Male patient, early 30’s, prior history of colon polyps
GBM removed by craniotomy 10 months ago, received temozolomide (TMZ)
Spinal metastasis resected 4 months ago, FMI test indicated high mutation load/pol E mutated germline status
Treatment with pembrolizumab initiated
Second spinal metastasis identified upon complications, removed 2 months ago
All tumors studied by high coverage exome sequencing compared to PBMC normal, and by IHC
GBM27: Clonal Evolution

[Graphs and diagrams showing clonal evolution across different stages and clusters.]
GBM27: Evolving Immune Response

- Patient remains on Pembrolizumab
- Recent MRI indicated response of remaining untreated right frontal horn lesion in the brain
**pVac-Seq Pipeline: Open Source Neoantigen Prediction**

- Exome sequencing data from tumor and normal are analyzed to identify somatic SNVs
- Each nonsynonymous SNV is converted to a mutant peptide sequence
- netMHC evaluates each mutant and wildtype peptide relative to the patient MHC haplotypes
- A list of peptides, predicted binding affinities and peptide identities is filtered to remove coverage-related false positives and alleles without evidence of RNA expression
- Indel prediction and translation now added to code base

**Hundal et al., Genome Medicine 2016**
Patient biopsied metastatic melanoma lesions

Tumor and germline DNA sequenced, somatic mutations identified

RNA seq verifies expressed mutations and expression level

netMHC algorithm identifies putative immunoepitopes

Apheresis samples from patient used for in vitro assays to refine the algorithmically-identified immunoepitopes (EliSpot and IFNγ)

Verified peptides are used in a patient-specific dendritic cell vaccine

Carreno et al., Science 2015
Genomic heterogeneity and RNAseq in vaccine design

Carreno et al., Science 2015
CD8+ T cell responses to mutation-containing peptides

Dextramer assays compare PBMC before vaccination and at peak post-vaccine administration to identify whether T cell populations specific for the peptides in the dendritic cell vaccine emerge post-vaccination.
Melanoma Dendritic Cell Vaccines: Conclusions

• Our first-in-human trial has demonstrated safety and a partial response of eliciting CD8+ T-cell memory for the tumor-unique neoantigens in three patients

• Verifying expression levels and allele-specific expression of mutations is critically important for neoantigen prediction, especially in heavily mutated tumor exomes

• The T cell repertoire in each patient was shown to be composed of diverse clonotypes post-vaccination

• We are presently working to improve the accuracy of the neoantigen pipeline predictions and to consider additional mutation types as potential neoantigens
pVac-Seq Pipeline: Remaining Challenges

Challenges:

- Want to predict neoantigens across the spectrum of variants (indels, fusions)
- Improve binding predictions for rarer HLA alleles
- Predict class II neoantigens
- Improve our prediction of which neoantigens are processed and presented to the MHC
- Screen predicted neoantigens to the SNP-corrected patient proteome
- Evaluate HLA mutations and their impact on HLA expression (RNA)

Hundal et al., Genome Medicine 2016
Conclusions

• Cancer genomics discovery is transitioning to clinical use

• Sharing information about genes, their alterations in cancer and correlation to therapy is increasingly important

• Cancer genomics also can inform immunotherapy decisions
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