Gaps in Translation of Immuno-Biomarkers into Clinical Laboratory Use

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The long road of translation of biomarkers into laboratory medicine

Job description

- Discovery is completely different from validation, and implementation in clinical laboratory testing
  - Discovery is scientifically, technologically driven
  - Validation is medically, regulatory driven
  - Implementation is customer, economically driven

Job responsibility

- Who should drive this process?
  - Scientist? – Do they understand the medicine? regulations? economics?
  - Physician? – Do they have time for basic science research?
  - Managers? – Besides the economics, do they understand everything else?
- Is there anyone that can do all of this?

Gaps in the long road of translation of biomarkers into laboratory medicine

Discovery
-Funding
-Validity

Validation
-Regulations
-Harmonization

Implementation
-Economics
-Marketing

Where is the money for discovery?

- Funding agencies request hypothesis-driven research for grant applications
- Biological hypothesis-driven ideas have little to do with biomarker discovery
- Thus, biomarkers discovery applications are not funded

- Once discovered, all biomarkers have biological explanations
- Thus, a good biomarker can drive biological hypothesis-driven research

- The money for biomarker discovery is coming from R&D budgets from manufacturers and reference laboratories

Validity: Are there enough subjects for discovery?

- Biomarker discovery must be made in patients
  - Must account for population diversity (e.g. ethnicity, genetics, HLA)
  - Disease biology, heterogeneity
- Multiple patient sets needed (sort of consensus from genomics/proteomics)
  - Discovery patient set: large cohort enough to account for all the above
  - Training patient set: independent cohort on which biomarker is refined
  - Test patient set: another independent set on which biomarker is validated
- Biomarker discovery might be stifled by hard realities of patient numbers
  - if you discover a biomarker test in 10 patients then you don't have a test
- Statistical power calculations come to the rescue

Regulations in the eye of the beholder (a.k.a.) FDA

- Categories of in-vitro diagnostic (IVD) assays
  - FDA-cleared: commercially distributed IVD assays approved for marketing and use in clinical laboratory diagnosis
  - Research Use Only (RUO): commercially distributed IVD assays in laboratory research phase of development; that is, either basic research or the initial search for potential clinical utility
  - Laboratory develop tests (LDT) – “home-brewed”: IVD assays that are manufactured, including being developed and validated, and offered, within a single laboratory using ASR. Traditionally developed by academic laboratories. More recently developed by whoever feels empowered (e.g. boutique testing). Until recently, FDA has used “enforcement discretion” to regulate LDT
  - Analyte Specific Reagents (ASR): reagents which, through specific binding or chemical reactions with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens

Understanding of regulations

- "Dear manufacturer" letter by FDA in June, 2011
  - Draft distributed for comments: Guidance on Commercially Distributed IVD Products Labeled for RUO (www.fda.gov)
  - Discourages manufacturers of RUO from selling their kits to customers that are using these products for clinical diagnostic use
  - In average RUO kits are used for ~30% of clinical laboratory test
  - Uncertainty about how FDA will implement, enforce this policy
  - Some manufacturers already starting to comply leaving clinical labs in the dark
  - A way out might be to buy kits as ASR's pieces and validate LDT's
  - What happens when LDT's become regulated by FDA?

Biomarker assay validation

- Parameters assessed in validation protocol (CLSI) for IVD assays
  - Accuracy
  - Reproducibility/ repeatability
  - Limit of detection/ limit of quantitation
  - Linearity
  - Potential interferences/ cross-reactivity
  - Matrix effect
  - Cross-contamination/ carry-over
  - Reference interval
  - …and a lot more specifically-related

Are we all reporting the same result?

- Science is reproducible but often laboratory testing is not
  - Protocol differences
  - Instrumentation changes
  - Human factor
  - Quality control issues
  - Normal assay reproducibility drift

- Harmonization ≈ proficiency testing programs are designed to address and correct reproducibility issues in clinical laboratory testing

- There is few harmonization data in laboratory validation and implementation of new immuno-biomarkers.

Are we all reporting the same result?

- Multicenter HLA-peptide multimer proficiency panel (MPP)
- The Cancer Vaccine Consortium of the Cancer Research Institute
- 27 laboratories tested two HLA-A2-restricted model antigens using commercially available HLA-peptide multimers

Table 1 Percentage of CD8-specific multimer binding based on the mean of the triplicates

Antigen	Donor	Median	25th Percentile	75th Percentile	Mean	SD	CV	Min	Max
Influenza-M1	D1	0.07	0.04	0.15	0.32	0.67	210.58[a]	0.00	2.87
	D2	0.67	0.50	0.89	0.73	0.34	47.16	0.24	2.05
	D3	0.16	0.11	0.32	0.29	0.35	122.95	0.00	1.53
	D4	0.19	0.17	0.27	0.28	0.26	93.66	0.07	1.44
	D5	0.35	0.31	0.41	0.44	0.25	57.05	0.21	1.21
Melan-A/Mart-1	D1	0.07	0.03	0.19	0.23	0.48	207.48[b]	0.00	2.53
	D2	0.10	0.03	0.17	0.17	0.27	156.14	0.00	1.30
	D3	0.12	0.06	0.23	0.25	0.40	157.76	0.02	2.09
	D4	0.10	0.04	0.15	0.17	0.23	135.44	0.01	1.06
	D5	0.08	0.03	0.17	0.18	0.26	142.63	0.01	1.06

Britten et al., Cancer Immunol Immunother 2009

Opportunities to improve among others…

- Establish lab SOP for MHC-peptide multimer staining
  - Count at least 100,000 CD8 T cells per staining
  - Introduce a background control to set gates
  - Use more than two colors for staining
- Establish SOP for software analyses
  - Gating strategy
  - Rules to set the gates
- Establish a human auditing process of all results
  - Are all dot plots correctly compensated?
  - Have the gates been set correctly?
  - Are the calculated frequencies of multimer-positive cells plausible?
- Use of dump channel, dead cell dyes, irrelevant multimers

Britten et al., Cancer Immunol Immunother 2009

Economics

- New biomarkers must be considered in the context of cost, throughput, and format availability
  - Central lab testing vs. point of care testing
  - Hospital-based vs. reference-based
  - Need for higher throughput technology?
  - Kits vs. ASR's
- Reimbursement costs
  - How much to charge for a new biomarker?
  - Volume? FDA-clearance?
  - Medicare, Medicaid
  - Insurances, out-off pocket
- Current trend for personalized medicine is in direct collision with health care reform

Marketing

- Do you know your clients? 3 P's: Physicians, Patients, Payors
- Physicians start a time clock after ordering a test; live in a world of complexity but demand simplicity; don't trust new biomarker tests
  - If you have a biomarker that takes 1 week to report or more than 1 minute to interpret then you don't have a test
- Patients know more about their symptoms, diagnosis, come to the office clinic demanding a list of laboratory tests (conventional, exoteric)
- All you need to know about what payors want from a new biomarker test is to save money or improve long-term health care…..and save money
  - If you have a biomarker that cost $1,000 then you don't have a test

Conclusions

- New immuno-biomarkers will change medical care and outcomes
- It is a challenging time for biomarker discovery, validation and implementation
- With new regulatory challenges come opportunities for those willing to embrace them
- The disconnection of expectations among physicians, patients, payors and health care reform policies needs to be bridged

It takes all of us