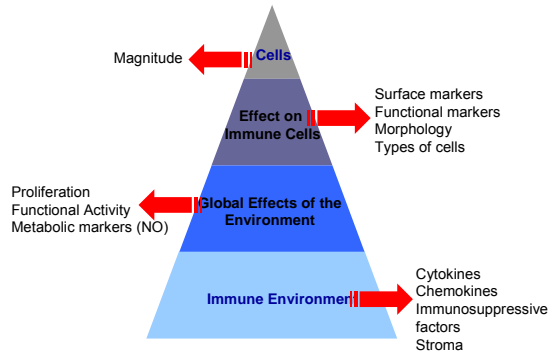


Immunologic Monitoring

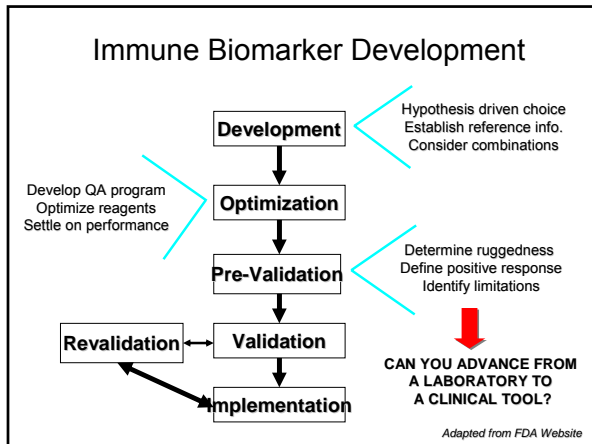
- I. Choosing an assay
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Immune Measurements that May Predict Clinical Outcomes

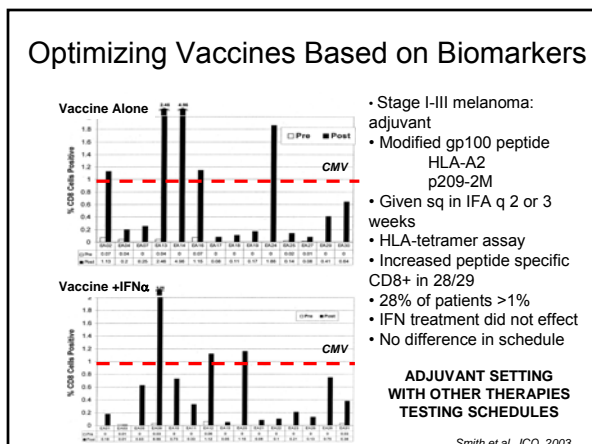


“Ideal” Clinical Immunologic Assay

- Uses minimal amount of clinical material
- Requires no *in vitro* expansion
- Multiple evaluations in short time period
- Sensitive to a broad range of responses
- Able to be used with cryopreserved cells
- Measures effector function
- Highly quantitative



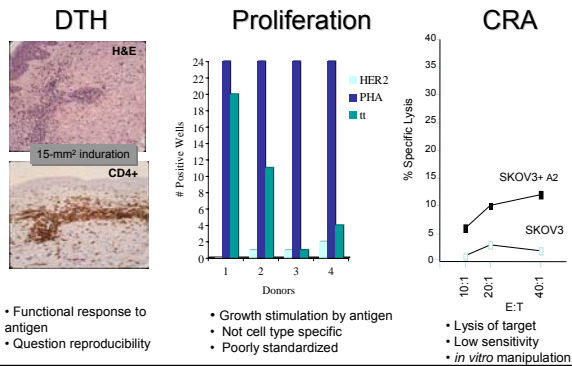
- ### Benefits of Assay Development
- Enumerate and characterize immune response
 - Assess potency of vaccine
 - Prioritize strategies and formulations
 - FDA interest for inclusion in clinical trials
 - Early biomarker of vaccine efficacy?



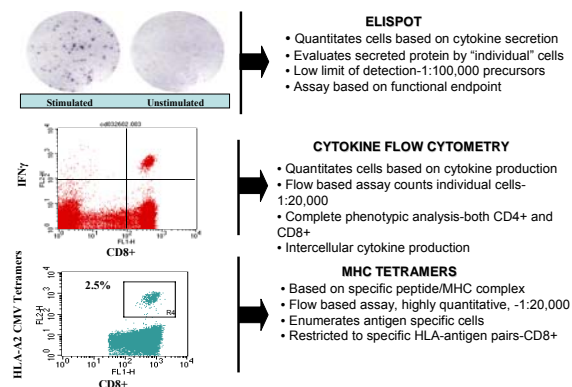
Functional and Quantitative Assays

- Blastogenic response to antigen
 - *Specificity a question*
- Delayed type hypersensitivity (DTH)
 - *Can assess function, not quantitative*
- Lytic activity
 - *Reproducibility a question, varying sensitivity*
- Cytokine secretion
 - *Quantitative and functional*
- MHC Tetramer analysis
 - *Highly quantitative, can be combined with functional assays*
- Antibody quantitation
 - *Humoral response, validated for infectious disease vaccines*

Qualitative Assays



Immunologic Biomarkers: CMV Immunity



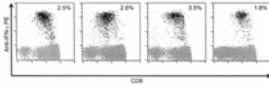
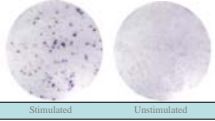
Cytokine Secretion

ELISPOT

- Low limit of detection
- Few cytokines
- No T cell subsets
- Prolonged *in vitro* step
- Technician dependent

Cytokine Flow Cytometry

- Higher limit of detection
- Several cytokines
- Multiple T cell subsets
- Short *in vitro* step
- More automated

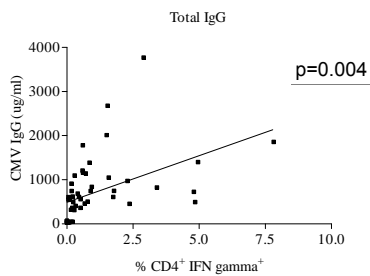


Smith et al., 2003, JCO

MHC Tetramers

- MHC-peptide complexes that bind Ag specific T cells
- Flow cytometric read-out
- No *in vitro* step
- Highly quantitative: not based on function (key for cancer)
- MHC restricted
- Can be combined with other assays

CMV Specific IgG Correlates with CMV IFN γ T Cell Response

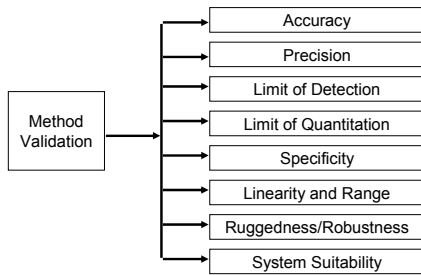


de la Rosa et al, U-54, 2004

Immunologic Monitoring

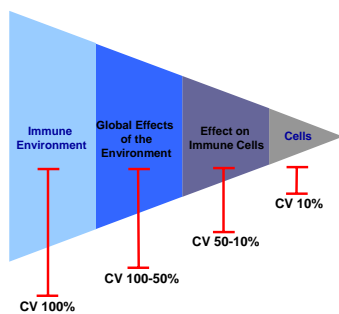
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Status of Immune Monitoring: Quantitation and Reproducibility

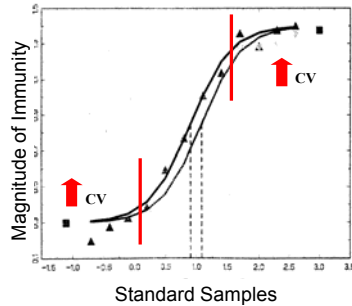


Dominique Pifat, FDA Website, 2003

Ability to Standardize Immune Measurements



Limit of *Reproducible* Detection



Challenges for Cellular Assays

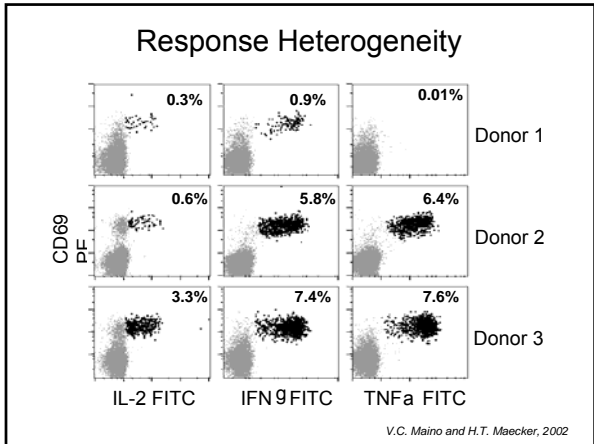
- Most sensitive may be least reproducible
- Establish performance
- Controls (Reference Pop):
 - Clones
 - Beads
 - Leukapheresis
- Low/Medium/High Response
- CMV as standard antigen
- Validation
 - New technicians
 - Reagent lot changes
 - Quarterly performance ✓

T Cell Assays

| Criteria | ELIspot | CFC | Tetramers |
|-------------------------------|--------------------|------------------|-------------------|
| In vitro stim | Yes | Yes | No |
| Time | >2 days | 1-2 days | Hours |
| Limit of detection | 1:30,000-1:300,000 | 1:5,000-1:20,000 | 1:10,000-1:50,000 |
| Loss with freezing | Yes | +/- | No |
| Capture cells for further use | No | Yes | Yes |
| Antigen specificity | Indirect | Indirect | Direct |

Lab Assay to Clinical Tool

| Parameters Evaluated | Research Assays HER2 Ab | Research Assays p53 Ab | CLIA Standard |
|----------------------|----------------------------|---------------------------|------------------|
| <i>Accuracy</i> | 12% | 10% | <10% |
| <i>Precision</i> | | | |
| Intra-assay | 9% | 12% | <10% |
| Inter-assay | 20% | 15% | <10% |
| <i>Specificity</i> | 77% | 100% | >80% |
| <i>Sensitivity</i> | 89% | 93% | >90% |
| <i>Linearity</i> | r=0.98 | r=0.95 | r=0.95 |



Immunologic Monitoring

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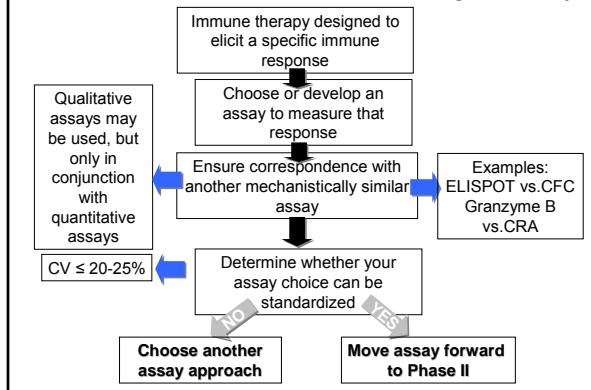
Laboratory Issues

- Develop screens for critical reagents (sera)
- Single lot or brand for entire study
- Uniform collection and storage
- Date reagents
- Reference population for validation (clones, lines, responder)
- Technical variation
- Establish assay range and QA program

Measuring Immunity to Predict Clinical Outcomes

- No known assay predicts outcome
 - Hypothesis driven
 - Pre-clinical data
- What you pick needs to be standardized
 - Test robustness early
 - Invest in QA
- Multi-parametric analysis may define the successful response
 - Patterns of response

Clinical Application of Immunologic Assays



References

Goodell, V. and Disis, M.L.: Human tumor cell lysates as a protein source for the detection of cancer antigen specific humoral immunity. *Journal of Immunologic Methods*. April 299:1-2:129-138, 2005.

Maecker, H.T., Moon, J., Bhatia, S., Ghanekar, S.A., Maino, V.C., Payne, J.K., Kuus-Reichel, K., Chang, J., Summers, A., Clay, T.M., Morse, M.A., Lyerly, H.K., delaRosa, C., Ankerst, D.P., and Disis, M.L.: Impact of cryopreservation on tetramer, cytokine flow cytometry, and ELISPOT assays. *BMC Immunology*. Jul 18; 6(1):17, 2005.

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Disis, M.L., Maecker, H.T., Clay, T.M., Lyerly, H.K., and Chang, J.C.C. Immunologic Monitoring to Assess Immunity to Solid Tumors in *Measuring Immunity: The Immunologic Surrogates Handbook*. First Edition" (eds) M.T. Lotze and A.W. Thomson. Academic Press, London, Chapter 40: 465-472, 2005.
