From eligibility to exploratory: planning and implementing biomarker testing for Immuno-oncology trials

Linda Robbie, Ph.D., Senior Director, Biomarkers and Global Translational Science Laboratory
Radha Krishnan, MD, Chief Pathologist and Senior Medical Director
Patrick Hurban, Ph.D., Senior Director and Global Head, Genomic Development/Esoteric Assays
Alistair J. Watt, Ph.D., Director, Translational Science Laboratory, Europe
The Opportunity for Immuno-Oncology (IO): The Future of Cancer Treatment

• Over 45 immuno-oncology drugs approved (US)*
• 57 immuno-oncology drugs in development*
• Over 250 studies registered/ongoing*
• Increasing number of relevant publications annually**

*UBS Immuno-oncology Monthly Handbook (Jan 2015)

**PubMed; keywords ‘cancer immunotherapy’
Novel Immunotherapies: Oncology’s “Breakthrough” Drugs

From hypothesis to realization and refinement

Examples of Immuno-oncology therapies

- Immunomodulatory cytokines/receptors (ex: OX40, CSF-1R)
- Bi-specific T cell engagers –BiTEs- 
- Chimeric antigen receptors (CARs)
- Checkpoint inhibitors (ex: CTLA4, PD-1/PD-L1)
- Anti-cancer vaccines
- Adoptive cell transfer
- Immunosuppressive metabolism inhibitors (ex: IDO)

TARGETED APPROACHES

www.researchcancerimmunotherapy.com
Classification of Immuno-Oncology Therapies

Immunomodulatory mAbs as Checkpoint Inhibitors: A Revolution

Figure 1: Anticancer Immunotherapy. Several anticancer immunotherapeutics have been developed during the last three decades, including tumor-targeting and immunomodulatory monoclonal antibodies (mAbs), dendritic cell (DC)-, peptide- and DNA-based anticancer vaccines; oncolytic viruses; pattern recognition receptor (PRR) agonists; immunostimulatory cytokines; immunomodulatory cell death inducers; inhibitors of immunosuppressive metabolism; and adoptive cell transfer. iMT, 1-methyltryptophan; APC, antigen-presenting cell; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IL, interleukin; IMiD, immunomodulatory drug; NLR, NOD-like receptor; TLR, Toll-like receptor.

Source: Galluzzi et al., Oncotarget, 5: www.impactjournals.com/oncotarget/
## Novel Immuno-Oncology Drugs in the Pipeline

<table>
<thead>
<tr>
<th>Developer</th>
<th>Molecule</th>
<th>Mode of action</th>
<th>Detailed mechanism</th>
<th>Indications</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate Pharma, Astrazeneca</td>
<td>IPH2201 (humanized IgG4 antibody)</td>
<td>NKG2A (CD94) inhibitor</td>
<td>Blocks inhibitory interaction between natural killer cell receptor NKG2A and its ligand human leukocyte antigen E (HLA-E), expressed on cancer cells</td>
<td>Head and neck cancer</td>
<td>Phase 2</td>
</tr>
<tr>
<td>BMS</td>
<td>Unetumab (BMS–663518; fully human IgG4 antibody)</td>
<td>4-1BB (CD137) agonist</td>
<td>Triggers co-stimulatory signal that promotes survival and expansion of activated CD8+ T cells and memory CD8+ T cells, as well as Tq, cytokine production</td>
<td>B-cell non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, multiple myeloma, head and neck cancer, colorectal cancer, non-small cell lung cancer, solid tumors</td>
<td>Phase 1 and phase 2</td>
</tr>
<tr>
<td>Pfizer Biomed</td>
<td>IMP321 (soluble fusion protein comprising four extracellular LAG3 domains fused to human IgG1 domain)</td>
<td>LAG3 agonist</td>
<td>Stimulates activation of antigen-presenting cells, resulting in CD8+ memory T-cell activation</td>
<td>First-line metastatic breast cancer, melanoma, prostate cancer, renal cell carcinoma</td>
<td>Phase 1/2a</td>
</tr>
<tr>
<td>AstraZeneca</td>
<td>MEDI6469 (CD134) agonist</td>
<td></td>
<td>Promotes survival and expansion of CD4+ Tq cells and CD8+ T cells</td>
<td>Advanced solid tumors, diffuse large cell lymphoma, head and neck cancer, prostate cancer</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>Novartis</td>
<td>LAG525 (humanized IgG4 antibody)</td>
<td>LAG3 inhibitor</td>
<td>Blocks inhibitory effect of LAG3 on activated T cells through MHC class II molecules</td>
<td>Solid tumors</td>
<td>Phase 1/2</td>
</tr>
<tr>
<td>arGEN-X</td>
<td>ARGX-110 (fully human IgG2 antibody)</td>
<td>CD70</td>
<td>Inhibits immunosuppressive interaction with CD27</td>
<td>Advanced CD70+ cancers</td>
<td>Phase 1b</td>
</tr>
<tr>
<td>Pfizer Biomed</td>
<td>PF-06082566 (fully humanized IgG2 antibody)</td>
<td>4-1BB (CD137) agonist</td>
<td>Triggers co-stimulatory signal that promotes survival and expansion of activated CD8+ T cells and memory CD8+ T cells, as well as Tq, cytokine production</td>
<td>Solid tumors, B-cell lymphomas, CD20+ non-Hodgkin’s lymphoma (in combination with Rituxan)</td>
<td>Phase 1</td>
</tr>
<tr>
<td>BMS</td>
<td>BMS–986016 (CD134) agonist</td>
<td>LAG3 antagonist</td>
<td>Blocks inhibitory effect of LAG3 on activated T cells through MHC class II molecules</td>
<td>Advanced solid tumors</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Calyvia Therapeutics (Hampton, New Jersey)</td>
<td>Varilumab (CDX-1127; fully human IgG1 antibody)</td>
<td>CD27 agonist</td>
<td>Induces T-cell activation and proliferation in presence of T-cell receptor stimulation</td>
<td>Solid tumors, hematologic malignancies</td>
<td>Phase 1</td>
</tr>
<tr>
<td>GITR, Inc. (Cambridge, Massachusetts)</td>
<td>TRX-518 (humanized IgG1 antibody)</td>
<td>Glucocorticoid-induced TNF receptor (GITR) agonist</td>
<td>Dampens immunosuppressive CD4+CD25+FOXP3+ Tq cells by inducing loss of FOXP3 expression</td>
<td>Advanced melanoma, solid tumors</td>
<td>Phase 1</td>
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<tr>
<td>Merck</td>
<td>MK-4166 (fully humanized IgG2 antibody)</td>
<td>GITR agonist</td>
<td>Dampens immunosuppressive CD4+CD25+FOXP3+ Tq cells by inducing loss of FOXP3 expression</td>
<td>Advanced solid tumors</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Jounce Therapeutics</td>
<td>Not disclosed</td>
<td>ICOS (inducible T-cell co-stimulator, CD278) agonist</td>
<td>Stimulates early expansion of CD4+ Tq cells and potentiates responses to CTLA-4 inhibition</td>
<td>Cancer</td>
<td>Proclinical</td>
</tr>
<tr>
<td>arGEN-X</td>
<td>ARGX-115 (camelid antibody)</td>
<td>GARP inhibitor</td>
<td>Blocks inhibition of immune cells by CD4+ Tq cells by preventing release of active TGF-β from GARP-TGF-β complexes</td>
<td>Cancer</td>
<td>Proclinical</td>
</tr>
<tr>
<td>Johnson &amp; Johnson</td>
<td>Antibody development program</td>
<td>V-domain immuno-globulin suppressor of T-cell activation (VISTA) antagonist</td>
<td>Disrupts VISTA-induced suppression of T-cell responses</td>
<td>Cancer</td>
<td>Proclinical</td>
</tr>
</tbody>
</table>

Sources: Company websites, clinicaltrials.gov, PubMed, Asco.org

Source: Nature Biotech. 33:7 Page 673-67, 52015
**Current Challenges**

Turning a fatal disease into a chronic treatable disorder

Over 800 cancer drugs in clinical trials - almost all are targeted at particular gene products

**Some Fundamental Problems in Cancer Treatment**

- **Cancer is rarely detected early**

- **Cancer develops resistance rapidly** to targeted therapies and chemotherapies due to the development/expansion of resistance mutations

- **Many patients do not respond to immunotherapies.** Immunotherapies are changing the approach to treating cancer by treating the immune system rather than the cancer but not all patients respond

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Response Rate</th>
<th>Toxicity</th>
<th>Long term Survival</th>
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</thead>
<tbody>
<tr>
<td>Chemotherapy / Radiation</td>
<td>Low</td>
<td>High</td>
<td>Poor</td>
</tr>
<tr>
<td>Targeted Therapy</td>
<td>High</td>
<td>Lower</td>
<td>Moderate</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>Mid</td>
<td>Lower</td>
<td>Good</td>
</tr>
</tbody>
</table>

*Source: Sharma, Allison: Cell, April 2015*
Immune-oncology: A Comprehensive Biomarker Approach is Needed

Tumor – Immune Biology
- Target protein expression
- Serum soluble proteins
- Circulating tumor cells
- Circulating free DNA
- Tumor gene expression
Immuno-oncology Research Requires Diverse Resources

An integrated scientific approach can help drive a successful I-O clinical study

Multiple Technologies and Methods

- Immuno-monitoring
- Target Occupancy
- Immuno-phenotyping
- Flow Cytometry
- Immunoassay
- Effector Function
- Cytokine Panels
- Tumor Lysates, Cell Sorting
- Digital Pathology
- ISH
- IHC
- Anatomic Pathology
- Genomics
- Tumor Antigens
- Immune Repertoire
- Gene Expression Profile

Integrated Scientific Teams

- Immunologists
- Geneticists
- Technical Experts
- Pathologists
- Clinical Scientists
- Bioinformaticists
Key Operational Challenges

Pre-Analytical Variables
- Tissue collection and fixation
- Sample processing
- Cell isolation
- Storage and shipment

Variation in Analytical Methods
- Multiple pathology scoring approaches and cutoffs
- FACS panel variability
- Variable PBMC/BMMC preparations

Limited Specimen Availability
- Exhausted or small biopsies
- Blood volume limitations restricting numerous analyses
- Inefficient sample usage
Anatomic Pathology
Anatomic Pathology and Immuno-Oncology
Predicting and assessing a patient’s response to checkpoint inhibitors

Samples
Routinely collected clinical samples

Central AP testing
Data can be mined for immune-oncology characterization

Potential Application
How these characterizations can be used in drug studies

Predictive Biomarkers for Optimized Patient Selection
CDx Registrational Trial

PD-L1 or related immuno-modulatory proteins
Tumor-infiltrating lymphocytes (TILs)
Image Analysis for multiplex IHC assays

Predictive Biomarkers for Optimized Patient Selection
CDx Registrational trial
Anatomic Pathology and Immuno-Oncology Clinical Trials

Ongoing biomarker assessments across all phases of development

- PD-1/PD-L1 expression by Immunohistochemistry (IHC) in multiple indications
  - Numerous assays and scoring methodologies
  - Expression on tumor cells vs. non-malignant cells
  - Cutoffs
  - Standardization of assay and scoring will be key
- Companion diagnostic (CDx) assay for PD-1/PD-L1 inhibitors
  - Central lab acts as investigative sites (PI and regulatory support)
- Tumor-infiltrating lymphocytes: Assay development for CD8, FoxP3 IHC
Key Challenge #1: Common Biomarker, Disparate Assays

Case Study: PD-L1

- High priority candidate biomarker for recent I-O studies
- Shared target in highly competitive space
- Multiple assays as candidate CDx assays for aPD-1/PD-L1 therapies
- Challenge for sponsors to ensure adherence to their specific assay and scoring criteria

Solutions and Success Factors:

- Connect Dx and central lab partners
  - Define parameters early
- Pathologist to pathologist discussion
  - Interactive discussion: Pathologists from pharma, Dx partner, and central lab early (+/- Ph 1)
  - Establish feasibility, mitigate risk, and potentially provide information to predict market uptake in “real world” for specific assay
- Initiate ring/concordance studies
  - Discuss early with pathologists and lab how to assess and track consistency in assay results
  - Establish best practices for training and testing with Dx and lab partners
  - Proficiency Testing
Key Challenge #2: Technical Variability in Sampling

Solutions to mitigate inconsistencies in sample processing and assay execution

- **Fresh tissue in fixative:**
  - Sample adequacy
  - Cold Ischemia
  - Type of fixative, time in fixative
  - Type of surgical procedure

- **FFPE (Formalin Fixed paraffin embedded) block:**
  - Availability of archival sample
  - Age of block
  - Archival versus fresh block
  - Adequacy of tissue/tumour in block
  - Megablocks
  - Selection of appropriate block

- **Glass slides used for section cutting and IHC staining**
  - Use of Superfrost slides
  - Labelling slides in sequence
  - Adequacy of tumour/tissue on sections
Protocol Review, Proactive Trouble-shooting, and Site Training to Promote Successful Outcome

- Upfront review of protocol considering indication, regional practices, available methodologies, and regulatory requirements (e.g. IVD, CLIA validation, etc.)
- Sample requirements per test and per patient
- Provision of standardized collection kits and supplies (e.g. slides, formalin, cassettes)
- Open communication and collaboration to link assay output with data and reporting requirements
- Site lab manuals and training

Case Study: H&E and IHC for enrollment

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Solution</th>
<th>Expected Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancellation rates 10-20% (H&amp;E) mostly due to lack of tumour in tissue submitted</td>
<td>Established AP Quality Management Plan</td>
<td>Improvement in cancellation rates to &lt;5%</td>
</tr>
<tr>
<td>• Delayed enrollment</td>
<td>• Capture metrics</td>
<td>Improved TAT</td>
</tr>
<tr>
<td>• Screen fails</td>
<td>• &gt;5% triggers investigation, sponsor notification, and site re-training</td>
<td></td>
</tr>
</tbody>
</table>

Q² Solutions
Key Challenge #3: Operations and Logistics

An essential component of the clinical biomarker testing strategy

• Logistics and TAT are often critical for patient enrollment

• Unforeseen factors can cause delays in testing and result in lost patients and reduced enrollment
  – Global differences in sample procession/prep
  – Import/export delays
  – Limited material
  – Communication and query resolution

• Planning and risk mitigation with laboratory operations can help prepare for ‘what-if’ scenarios
  – Incorporate discussions with laboratory, pathologist, and project team prior to study launch
  – Establish communication plan and POC
  – Address the details

Most central laboratories have a broad perspective across successful and less successful strategies. Engage their expertise.
# Companion Diagnostic Development Flexibility

Flexible laboratory solutions to support test development and trial execution

<table>
<thead>
<tr>
<th>Situation</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Large pharma required flexible solutions for PD-L1 IHC assay development in early-phase studies with late-phase study support</td>
<td>• Staffing provided to meet project timelines and budget</td>
</tr>
<tr>
<td>• Partnership with major Dx company for IVD kits, submissions and commercialization</td>
<td>• Pathologist/AP director was responsible for IHC test scoring methods and assumed Dx company responsibilities for some indications</td>
</tr>
<tr>
<td>• Responsibilities for assay development varied by indication depending on Dx company willingness to assume risk of future diagnostic product</td>
<td>• Supported design control at Dx partner</td>
</tr>
<tr>
<td>• Staining and scoring methods varied, requiring close collaboration and communications</td>
<td>• Leveraged relationship with diagnostic company and established methods of work to improve project efficiencies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Flexible assay development services to match sponsor and Dx company requirements enabled sponsor to obtain prototype tests for a wider range of indications and programs than what would have been available from the Dx company</td>
</tr>
<tr>
<td>• PD-L1 assays available for multiple sponsor programs, matching performance requirements as well as documentation for future regulatory submissions</td>
</tr>
</tbody>
</table>
**Case Study: Pathologist Expertise for IHC CDx**

*Development of scoring methodology for Rx-sponsored study using Dx-provided assay*

**Situation:** Pharma requires pathologist expertise and central testing for large CDx program

**Solution:**
- Pathologist familiar with drug MOA engaged to score and refine protocols for multiple indications in collaboration with Rx pathologist and support from Dx
- Global laboratories trained with expanded pathologist team led by lead pathologist

**Result:** Global assay deployment, cost and time-savings for pharma, central lab pathologist support for regulatory discussions

*Q² Solutions refers to either legacy Quintiles or legacy Quest central laboratories*
Genomics
**Genomics in Immuno-Oncology Innovation**

New opportunities for molecular characterization

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**Samples**
Routinely collected clinical samples

**Analysis**
Data can be mined for molecular immune-oncology characterization

**Potential Application**
How these characterizations can be used in drug studies

---

**Self-recognition**
HLA and KIR genotyping

**Immune activation**
B and T-cell repertoire, Immune gene signature

**Tumor characterization**
DNA and RNA

---

Exploitation of Innate and Adaptive Immune Response to Tumors

Optimized Patient Selection Refinement of Immuno-modulatory Therapies

Cancer Vaccines and Tumor-Specific Immune Responses
Personalized/ Precision Approach

Need to match the patients to them

Patient – Tumor Profile
- Transcriptome – RNA-Seq
- Serum proteins – proteomics
- Genomics– NGS
- RNA transcriptome – RNA-Seq

Patient genomic pre-profiling is a paradigm shift leading to better match patients to a drug treatment which best fit their conditions
Genomic Pre-Profiling
Benefits for patients and Pharma

• Higher actionability increases patient and physician interest
  – General oncology test can identify many pan-cancer biomarkers (~2-3wks)
  – Increases chance for rare positive biomarkers to be found for specific trials

• Enhanced Trial enrollment
  – Large sample study shows that 11% (of 2000) went onto genotype-matched trials
  – Many Groups facilitate this matching
    • Molecular Match, Caris, IBM (Watson)

• Personalized arms using genomic biomarkers had increased Overall Survival / relapse-free survival over standard protein over-expression assays

• New Protocols leverage this capability
  – TAPUR (ASCO) M-PACT (NCI)
  – Lung-MAP (NCI) IMPACT-2 (MD Anderson)
  – FOCUS-4 (UK)

• Future Longitudinal data will show how these matched therapies have performed

**Novel Antigen Determination Can Kick-Start Immunity**

- In most cases, somatic mutation calling from Exomes provides early evidence of malformed antigens.
- Coupling with RNA-Seq is essential to confer expression of that variant allele.

- Clinical group groups by mutation burden within antigens.
- Creating custom antibodies to restore recognition has increased survivability.

*Source: Van Allen et. al. (2015) Science 350(6257):207*
**Immune Repertoire has Prognostic Value**

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>ORR%</th>
<th>CR%</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapsed/Refractory [23]</td>
<td>85</td>
<td>71</td>
<td>4</td>
<td>75% at 26 months</td>
</tr>
<tr>
<td>Treatment Naive³</td>
<td>31</td>
<td>74</td>
<td>10</td>
<td>96% at 15 months</td>
</tr>
<tr>
<td>High Risk¹</td>
<td>24</td>
<td>59</td>
<td>0</td>
<td>NR</td>
</tr>
<tr>
<td>High Risk²</td>
<td>29</td>
<td>53</td>
<td>NR</td>
<td>85% at 20 months</td>
</tr>
</tbody>
</table>

**Combinations:**
- Ibrutinib + Ofatumumab⁵ | 27 | 100 | 4 | 89% on study at 10 months |
- Ibrutinib + FCr² | 3 | 100 | 67 | 100% at 11 months |
- Ibrutinib + Rituximab⁴ | 40 | 85 | 3 | NR |
- Ibrutinib + BR² | 30 | 93 | 13 | 90% at 11 months |

- IGVH typing confers relative health of cellular receptors
- Patients effectively respond to Ibrutinib when IGVH type = “unmutated”
- TCRs are obviously affected in response to disease and provide potential targets for therapies (CAR-T)
- Luo et al also shows improved Erbitux response for patients with appropriate TCR repertoire

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**Source:** Dias et al. (2016) *Cardiovascular & Hematological Agents in Medicinal Chemistry* 11.4 : 265–271

**Source:** Ruggerio et al. (2015) *Nature Communications* 6. 801
Genomics Capabilities for Immune Oncology

Analyzing the tumor: immune system interface

**Mutation Analysis**
- Whole-exome deep sequencing to quantify mutational burden
- Identification of ‘neo-antigens’
  - Coupled with RNAseq to confirm tumor antigen expression
- BFX and predictive algorithms to generate ‘immunogenicity score’
- DNA damage and mismatch repair:
  - MSI instability (PCR and IHC)
  - BRCA1 mutation analysis

**HLA and KIR Genotyping**
- HLA allele calling from:
  - Microarray
  - RNAseq
  - Targeted DNA sequencing
- KIR genotyping and expression (from PAXgene) using commercial kit (Miltenyi)

**Immune Repertoire**
- TCR (α/β, γ/δ) and BCR receptor sequencing to assess clonal diversity*
- IgVH mutation analysis (RNA-based)

*In development; planned for 2016

Source: Draper et al, Clinical Cancer Research. October 1, 2015

http://www.innovations-report.com
**Immuno-oncology is Genomics Solutions Oriented**

*Molecular methods provide an advantage for complex biomarker analysis*

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Genomics Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Somatic Mutation Analysis</strong></td>
<td><strong>Tumor Exome Sequencing, Breakpoint analysis</strong></td>
</tr>
<tr>
<td><em>Identify Neo-Antigens</em></td>
<td><strong>and Fusion Detection</strong></td>
</tr>
<tr>
<td><strong>Gene Expression Profiling</strong></td>
<td><strong>Pan-Cancer Immune Panel, RNA-Seq, Arrays</strong></td>
</tr>
<tr>
<td><em>Confirm Neo-Antigen Expression, Identify</em>*</td>
<td></td>
</tr>
<tr>
<td><strong>Immune Activation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Germ Line Variant Analysis</strong></td>
<td><strong>CNV, Structural and Small Variant Detection</strong></td>
</tr>
<tr>
<td><em>Confirm Somatic Mutations and Heritable</em>*</td>
<td></td>
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<tr>
<td><strong>Lesions</strong></td>
<td></td>
</tr>
<tr>
<td><strong>HLA Characterization</strong></td>
<td><strong>HLA Analysis (DNA, RNA, Arrays)</strong></td>
</tr>
<tr>
<td><em>Identify Mutations in HLA</em>*</td>
<td></td>
</tr>
<tr>
<td><strong>Antigen-Specific Immune Response</strong></td>
<td><strong>B/T Cell Repertoire (DNA/RNA),</strong></td>
</tr>
<tr>
<td><em>Characterize Immune Activation</em>*</td>
<td><strong>VDJ mutation (DNA/RNA)</strong></td>
</tr>
</tbody>
</table>
Summary

Improved response from patients is coming

• Genomic panels facilitate therapeutic approaches based on individualized mutation status

• Targeted Therapies have improved response, but many individuals do not benefit from this approach

• Immunotherapies can produce a very strong response but individualizing to a patient is complex
  – Multiple assay types
  – Multiple mechanisms to profile

• Genomic Assays provide molecular insight into the genetic make-up of a tumor and the character of the immune response

Taking advantage of this approach can leverage existing sample collection methods
Flow Cytometry
Flow Cytometry in Immuno-Oncology

New opportunities for immune characterization

Samples
Routinely collected clinical samples

Central Flow Analysis
Data can be mined for immune-oncology characterization

Potential Application
How these characterizations can be used in drug studies

Immune Status
- T, B, NK, Monocyte, DC
Cell Subset Activation Profile

Receptor Occupancy
ExVivo Predictive Assay

Predictive Biomarkers for Optimized Patient Selection
Correlation of Activation Profile to Response
Retrospective Data Analysis

Blood

Q² Solutions
State of the Art Flow Cytometry
Assay Development, Validation and Deployment Globally

- 8 & 10 Color BD FACS Canto Instruments
- >40 flow panels validated and transferred in 2015
- Typically 8-10 weeks per validation
- >100,000 flow samples analyzed in 2015
Flow Cytometry Assay Validation

- **Preliminary Work**
  - Panel design
  - QC material selection
  - Sample type
  - Gating Strategy

- **Validation**
  - QC Precision
    - Sets acceptance criteria for daily QC run
  - Sample Precision
    - 6 samples in triplicate (mean, %CV)
  - Stability
    - 6 samples 4-7 days
  - Custom
    - Stability/Precision in Spiked or Diseased Samples
    - Receptor Occupancy
    - Interference
    - Linearity/Sensitivity

<table>
<thead>
<tr>
<th>Tube</th>
<th>Fluorochrome</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CD197</td>
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<td></td>
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<td>CD45RA</td>
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</table>

**Graph:**
- Mean NaHep
- Mean ACD
- Mean EDTA
- Mean CytoChex

**Table:**

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<thead>
<tr>
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<tbody>
<tr>
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<td>CD45RA</td>
</tr>
<tr>
<td>2</td>
<td>CD45RA</td>
</tr>
<tr>
<td></td>
<td>/</td>
</tr>
<tr>
<td>1</td>
<td>CD8</td>
</tr>
<tr>
<td>2</td>
<td>CD8</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
</tr>
<tr>
<td></td>
<td>CD4</td>
</tr>
</tbody>
</table>
Stability/Precision in Spiked Samples

A263 T Cell Subset Proliferation (Ki67)

**CD8+Ki67+ Normal Donor Samples**

<table>
<thead>
<tr>
<th>CD8</th>
<th>Ki67</th>
<th>AP</th>
<th>CD9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Precision**

| Average SD | 0.3 |
| Average %CV | 22.9 |

**Stability**

<table>
<thead>
<tr>
<th>Mean</th>
<th>1.1</th>
<th>1.1</th>
<th>1.0</th>
<th>1.0</th>
<th>0.9</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Change from Day 0</td>
<td>23.5</td>
<td>11.0</td>
<td>19.0</td>
<td>16.8</td>
<td></td>
</tr>
</tbody>
</table>

**CD8+Ki67+ Spiked Donor Samples**

<table>
<thead>
<tr>
<th>CD8</th>
<th>Ki67</th>
<th>AP</th>
<th>CD9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15.1</td>
</tr>
</tbody>
</table>

**Precision**

| Average SD | 1.4 |
| Average %CV | 6.4 |

**Stability**

<table>
<thead>
<tr>
<th>Mean</th>
<th>17.9</th>
<th>18.6</th>
<th>19.2</th>
<th>19.1</th>
<th>18.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Change from Day 0</td>
<td>10.8</td>
<td>9.3</td>
<td>13.0</td>
<td>9.9</td>
<td></td>
</tr>
</tbody>
</table>

---

**Q² Solutions**
**Receptor Occupancy**

- Different strategies available

- Validation
  - Dose Response Confirmation
  - Precision at approx. 100/50/0% saturation
  - Stability at approx. 100/50/0% saturation

- Receptor Occupancy experience at Q² Solutions
  - CD28 - CD30
  - PDL1 - CD4
  - CD86 - CD30
Flow Cytometry Technology Transfer

• **Split sample method comparison**
  - Dependent on stability of assay

• **QC Precision**
  - Range confirmation against TSL

• **Sample Precision**
  - Comparable precision to TSL

• **Gating Assessment**
Flow Cytometry Instrument & Assay Standardization

- **Instrument Standardization**
  - Standardized Quantitative Instrument Setup
    - Standardized against a composite predicate instrument using FCB targets
  - Applications Settings & Embedded Reusable Spillover
  - Q² Solutions Instrument Performance Specifications
    - Superior to BD manufacturing & service specs
  - Intra/Inter Instrument Performance Tracking
    - Trends are tracked daily by Global Harmonization
    - Identify potential problems and prevent downtime

- **Assay Standardization**
  - Presentation & SOP
  - Gating Guides
  - QC
    - Defined during validation
    - Global QC ranges/ harmonized QC lots
    - Central QC data tracking
  - Analyst Proficiency Testing
  - Central Gating
Q² Flow Panels Identify Dose Dependent PD Effect of Checkpoint Inhibitor Combination Therapy

MEDI4736 (anti-PD-L1) & Tremelimumab (anti-CTLA-4)

Combined data from A115 & A129 Flow Panels running in US (Atlanta), Europe and Asia (Singapore)

Source: Safety and antitumour activity of durvalumab plus Tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study
Scott Antonia, Sarah B Goldberg, Ani Balmanoukian, Jamie E Chaft, Rachel E Sanborn, Ashok Gupta, Rajesh Narwal, Keith Steele, Yu Gu, Joyson J Karakunnel, Naiyer A Rizvi
Lancet Oncology 2016. Published February 5, 2016
Flow Cytometry Panel Development in IO
Considerations

• Early engagement
• Focussed parameter list
• Custom panel design
• Tube type selection for maximum sample stability
• Ongoing data review
Conclusions
**Potential Roadmap for Biomarker Strategy**

*Multiple parallel and synergistic pathways maximizing immediate lab opportunities as well as long term clinical differentiation*

**Prioritized Biomarker Activities**
1. Develop next-gen IHC tests
2. RNA-Seq – gene expression analysis
3. Immunoassay serum protein proteomics
4. Flow cytometry immune cell population profiling
5. Genomic profiling for DNA repair defects/mutation load and microbiome profile

**Diagram**
- Non-biomarker research & bioinformatics
- Preclinical & Clinical Research
- Patient Samples & Clinical Data
- Genomic profiling
- Microbiome
- RNA-Seq
- Flow cellular profiling
- TIL Analysis
- IHC Test
- Serum proteomics
- NGS IO CDx
- Immunoscore
- Multiplex IHC Test
- Response Signature
- Immunoassay Test
- Nanostring Test
- Patient Monitoring
- Novel Drug Comb.’s

**Opportunities for synergies**
Precision Medicine
Partner of choice for CDx development from biomarker ID to CDx launch