Molecular Profiling of Tumor Microenvironment

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Cellecta, Inc.
Cellecta, Inc.  
- Founded: April 2006
- Headquarters: Mountain View, CA
- 12 SBIR Grants
- Custom Service Provider for Functional Genomics

Portfolio  
- Genetic Screening Services - CRISPR & RNAi
- sgRNA & shRNA Libraries (Custom & Premade)
- Isogenic Cell Line Services (Custom)
- Next-Gen Sequencing & Analysis
  - Driver-Map™ Targeted RNA Expression
Systems Biology of Cancer

**Challenges**
- Heterogeneity
- Directed & Stochastic evolution
- Plasticity
- Reprogramming immune & stromal cells

**Causes**
- Genetic backgrounds & Lesions
- Epigenetic changes
- Homeostasis, Environment (age, microbiome, stress, infection / inflammation, etc.)

**Unmet Clinical Needs**
- Resistance to targeted agent
- Toxicity of chemo & drug combinations
- Diagnostic & Prevention of metastatic disease
- Personalized therapy

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Microenvironment Role in Cancer Hallmarks

Unique Expression Profile of Blood Cells

Immortalized Cells

Tissues

Whole Blood

Data: GTEx (Genotype-Tissue Expression Project)
Profiling of Immune Cell Subtypes

RNA-Seq profiling data for appr. 1,600 genes can be accurately clustered into 38 modules, including naïve and memory B, natural killer (NK), dendritic and several T-cell subtypes.
Profiling of Immune Cells in Tumors

TCGA expression Data

Development of Classifier Model for Different Cell Types in Tumor Microenvironment
Objectives:

• Develop CancerNet™ expression assay for analysis of cellular composition in complex TME

• Molecular profiling of key immune-related genes, including drug targets, known biomarkers, and immune mechanisms

• Unbiased discovery of most informative biomarkers that can be analyzed by conventional IHC/FACS or Q-RT-PCR assays

CancerNet 8K Assay Gene Composition

- **8 Cancer Hallmarks**: 4,000
- **Pathways & Signaling Networks**: 5,600
- **Drug Metabolism**: 300
- **Drug Targets (FDA, Clinical trials)**: 3,700
- **Extracellular proteome (secreted proteins, receptors, ligands)**: 900
- **Pan-Cancer Drivers**: 1,600
- **Cell Lineage Signatures**: 1,000
- **Immunity Mechanisms & Immunotherapy Markers/Targets**: 550
- **TME 2.5K**:
  - **Tumor Microenvironment (TME) Profiling**: 1,600

**Total**: 8,250 genes

**CancerNet™ 8K**

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Targeted RNA Expression (TREx) Technology

- Multiplex RT-PCR-NGS assay for expression profiling of up to 20,000 genes in a single test-tube assay (no intermediate purification steps)
- Requires 10-100ng of total RNA from biopsy, whole blood, and PFPE/(FFPE) samples
- Built-in calibration standards for QC and normalization of expression data
CancerNet for Cell Type Profiling

Samples
- Tumor
- Blood
- (FFPE?)

Targeted RNA Expression Profile

Targeted RNA expression profile of 8,000 genes
Detect presence of immune cells using reference cell lineage-specific signatures
Enumerate immune/stromal cell types using cell-specific calibration standards

Cell Composition Profiling

Stromal Cells
- Endothelial
- Fibroblast

Cancer Cells
- Stroma
- Cancer Cell
- T Cell
- Macrophage

Immune Cells
- B Cell
- NK Cell

Relative Fraction - and - Enrichment Score

Cell Populations in Tumor Microenvironment
Comparison of Gene Expression Technologies

- **Targeted RNA Expression (TREx)**
  - Dynamic Range (Expression Level) 100,000 to 10,000
  - # of Expressed Genes 25,000 to 10

- **NanoString**
  - Dynamic Range (Expression Level) 1,000 to 100
  - # of Expressed Genes 5,000 to 10

- **Raindance**
  - Dynamic Range (Expression Level) 100 to 10
  - # of Expressed Genes 1,000 to 10

- **RNA-Seq**
  - Dynamic Range (Expression Level) 1,000 to 100
  - # of Expressed Genes 20,000 to 1,000

- **Microarrays**
  - Dynamic Range (Expression Level) 1,000 to 100
  - # of Expressed Genes 20,000 to 1,000

Single RT-PCR → Multiplex RT-PCR
Low Sensitivity Expression Profiling
High Sensitivity Expression Profiling
Heat Map of RNA-Seq and TREx Data

RNA-Seq

CancerNet 8K

Highly Abundant

Medium Abundant

Low Abundant

Reads (log2)

20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Comparison of TREx and RNA-Seq

- CancerNet 8K shows 100-fold increase in sensitivity
- ~2-fold increase in number of detected genes (> 10 reads/gene)

MDA-MB231-100 ng/1 ug
Development of Functionally Validated RT-PCR Primers

- Functional validation of 5-12 primer pairs per gene in highly multiplexed PCR (x 1,000's primers) assay using set of synthetic and universal RNAs

- Human-specific PCR primers for profiling in xenograft mouse model

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Unique Primer Design Prevent Primer Dimers
Selection of Primers with Highest Sensitivity

Human Universal RNA

- #1
- #2
- #3
- #4

Primer Set: 1 2 3 4 5

CD55 TLR4 IGF1R

BEST

# of reads
Selection of Primers with Highest Specificity
Primers for Conservative cDNA Regions

Human Universal RNA
- #1
- #2
- #3
- #4

# of reads

Primer Set:
- MS4A6A
- C5

isoform-specific

BEST
Primers for Highly Abundant HK Genes

# of reads

Primer Set: 1 2 3 4 5 6 1 2 3 4 5 6 7

GNAS  GAPDH

Human Universal RNA

Too high

Too high

BEST

BEST

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TREx Technology Advantages

- Genome-wide multiplex RT-PCR amplification of up to 20,000 known genes (conservative cDNA regions) followed by quantitative analysis of expression data by NGS (Illumina)
- Single test-tube protocol with highly validated RT-PCR primers starting from 1-10ng of total RNA (whole blood, PBMC, biopsy, xenograft, PFPE (FFPE?))
- Digital expression profiling in $10^5$-fold dynamic range with zero background
- 100-fold increase in sensitivity and 2-fold increase in the number of quantitated expressed genes in comparison with conventional stranded RNA-Seq protocol
CancerNet 8K Assay Gene Composition

- 8,000 Genes
  - 4,000 Cancer Hallmarks
  - 3,700 Extracellular proteome (secreted proteins, receptors, ligands)
  - 900 Drug Targets (FDA, Clinical trials)
  - 1,600 Pan-Cancer Drivers
  - 1,000 Immunity Mechanisms & Immunotherapy Markers/Targets
  - 1,300 Pathways & Signaling Networks
  - 550 Cell Lineage Signatures
  - 500 Drug Metabolism
  - TME 2.5K Tumor Microenvironment (TME) Profiling

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Profiling of Immune Cell Subtypes

TME profiling data can be accurately clustered into 28 modules, including naïve and memory B, natural killer (NK), dendritic and several T-cell subtypes.
Sensitivity of T Cell Detection in Tumor

Cytotoxic T Cell : Tumor Admixtures

Detection Limit 0.5%

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Reproducibility and RNA Requirement

High Reproducibility in the range of 10ng-100ng of Total RNA
Sample Requirement

1-100 ng Total RNA for Tissue, Blood, PFPE

Frozen tissue (Biopsy, FNA, Xenograft)

Blood (Whole, PBMC)

PFPE (FFPE)

CancerNet 8K

50-100ng FFPE if pass QC for size (>300-n) and % of functional RNAs (>10%)
CancerNet 8K Profiling in Tumor Samples

Highly infiltrated tumor cells
BREAST CANCER PATIENT 166

Low infiltrated tumor
Patient 170

Differential Gene Expression Analysis

Rep#: 1 2 3
Infiltration: n/a + +++
Sample: Normal Tissue Breast Cancer Patient 1 Breast Cancer Patient 2

ADORA2A
BATF
BTLA
CCL13
CCL17
CCL22
CCR2
CCR3
CD19
CD1A
CD1B
CD2
CD27
CD3B
CD40LG
CD6
CD8B
CR2
CXCR6
EWSR1
FOXP3
FUT5
GATA3
GNLY
GZMB
HAVCR2
HLA-DOB
ID01
IL21R
KIR3DL1
LAG3
LAIR2
LTA
LTK
MAGEA1
MAGEA12
MAGEA3
MAGEA4
MAGEB2
MAGEC1
MAGEC2
NCR1
PCD1
PRAME
REPS1
SEMG1
STAT6
TBX21
TNFRSF4
TNFRSF9
### Profiling Immune Cells with Lineage-Specific Markers

**IMMUNE CELL INfiltration:**

- **166N**
  - [+++++]

- **170N**
  - [+]

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**Image Description**

- **IMMUNE CELLS**
  - **TUMOR**

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**Heatmap**

- **Row annotation**
  - b-cell
  - B-cell
  - B-cell
  - CD8 T-cell
  - CD8 T-cell
  - Checkpoint
  - Checkpoint
  - Checkpoint
  - Checkpoint
  - Cytotoxic cell
  - Cytotoxic cell
  - DC
  - DC
  - DC
  - Macrophages
  - Macrophages
  - Macrophages
  - Mast cell
  - Mast cell
  - NK CD56dim cell
  - NK cell
  - NK cell
  - T helper cell
  - T helper cell
  - T helper cell
  - T-cell
  - T-cell
  - TFH
  - TFH
  - TFH
  - Tcm
  - Tcm
  - Tem
  - Tem
  - Th1 cell
  - Th1 cell
  - Th1 cell
  - Th1 cell
  - Th1 cell
  - Th17 cell
  - Th2 cell
  - Th2 cell
  - Treg
  - aDC
  - aDC
  - aDC
  - IDC
  - IDC
  - IDC

**Color Scale**

- **Legend**
  - Blue to yellow gradient
  - Range: -2.0 to 2.0

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- [Logo]
Gene Set Enrichment Analysis of CancerNet 8K Data

Infiltrated Tumor

Gene

Custom Service Report

Basic Service
1. Expression levels and annotation for the 8K genes
2. Immune Cell subtypes detected: Leukocytes (T cells (CD4, CD8, Th1, Th2, Th17 and Treg), B cells, NK/NKT cells, Macrophages, neutrophils, dendritic cells, stromal)

Extended Service
1. Accurate enumeration of cell composition requires calibration experiment with cell-specific cell RNAs spiked in the RNA isolated from specific tumor type (background)

http://www.driver-map.com/immunenet/
CancerNet 8K for TME Profiling

- Infer tumor infiltrating immune cell types & immune/stromal/cancer cell composition
- Profile the immune response in tumors
- Quantitate expression levels of drug targets
- Monitor patient response to drug treatment
- Discover prognostic/predictive biomarkers
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<th>Discovery / Profiling</th>
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<td>Generic gene discovery</td>
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<td>Pan-cancer driver genes</td>
<td>Mutations in both DNA &amp; RNA</td>
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Development of Validated Custom Assays

- Genome-Wide GeneNet 18K Primers (Human & Mouse)
- CancerNet 8K Assay
- Custom Targeted RNA Expression Assay 100 - 5,000 genes

Discovery of Key Biomarkers

Q-RT-PCR Assay Development for Clinical Research

Assay Development & Validation

HT Profiling of Clinical Samples

Selection of the Most Informative Biomarkers
Key Features of Driver-Map Portfolio

- Premade, custom kits and services for targeted RNA-Seq expression profiling with genome-wide and focused gene panels
- Single test-tube, multiplexed RT-PCR-NGS with up to 96 clinical samples in a single sequencing run
- Quantitative expression data in $10^5$-fold dynamic range starting from 10 ng of total RNA (Blood, Tissue, Xenograft)
- Employs synthetic competitive template internal standards designed for all targeted amplicons
- Direct validation and analysis of most informative biomarkers by conventional qRT-PCR for clinical research
Acknowledgments

- Gus Frangou – Cellecta
- Michael Makhanov – Cellecta
- Sunitha Sastry – Cellecta
- Leonid Iakoubov – Cellecta

Thank you!