Immunophenotyping to Differentiate Responder and Non-responder Patients in Cancer Immunotherapy

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Disclosures

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The Promise of Immuno-therapy
Cancer Immunotherapy

- significant variation in response rates between and within tumor types
- need for more sophisticated immunophenotypic methods for predictive and prognostic profiling of responder (R) and non-responder (NR) patients
- high cost of futile therapy in NR cohort plus indirect care costs for management of toxicity / AEs
- need for more informed rationale for combination regimens
  - doses, sequence
  - Rx classes
Deconvolution of the Complex, Multi-Dimensional Matrix of Immuno-Oncology Therapeutics
Deconvolution of the Complex, Multi-Dimensional Matrix of Immuno-Oncology Therapeutics
Relationship of Tumor Burden, PFS and OS to Enhanced Expression of Ki67 as CD8⁺ Proliferation Marker in Pembrolizumab Anti-PD-1 Therapy of Stage IV Melanoma (n=23)

From: A. C. Huang et al. Nature (2017) doi:10.1038/nature22079 Supplemental Data Fig. 9
Heatmap of Differentially Expressed Genes in Tumor Infiltrating Lymphocytes and Identification of Activation and Dysfunction (Exhaustion) Modules

M. Singer et al.(2016) Cell 166, 1500
The Complex Metabolic Landscape of the TME and Potential Effects on Anti-Tumor Immune Functions

- competition of tumor cells and activated immune cells for tryptophan, arginine and glutamine
- over expression of indoleamine 2, 3-dioxygenase (IDO) by tumor cells
  - tryptophan metabolites (e.g. kynurenines) down regulate TCR and induce FOXP3^+ Tregs
- arginine depletion
  - impaired T cell function, induce MDSC generation
- glutamine depletion
  - promotes generation of Tregs
- CD73 expression on tumor cells and adenosine accumulation
  - impaired T and NK cell activity, recruitment of MDSC
Large Scale Profiling of Markers Relevant To Immune Checkpoint Blockade Therapy

- 120,000 Cancer Patients Profiled
- Programmed Cell Death-Ligand 1 (PD-L1) Immunohistochemistry 46,000+ Tests Performed
- Microsatellite Instability (MSI) Next-Generation Sequencing 21,000+ Tests Performed
- Total Mutational Load (TML) Next-Generation Sequencing 17,000+ Tests Performed
Frequency of MSI-High Profiled by NGS in 31 Cancers (n = 11, 251)
Scatter plots comparing MSI determined by NGS and TML per megabase for colorectal adenocarcinoma (n = 1267), endometrial cancer (n = 667), NSCLC (n = 964), and melanoma (n = 175)
Overlap of TML, MSI and PD-L1 Markers in 2,918 Patients
In Cohort of Total 11,780 Patients

TML n=876
MSI n=342

PDL1 n=1700

Triple negative n=8952

TML

MSI

PDL1

408

194

93

46

228

9

1417
Overlap of TML, MSI and PD-L1 Markers in Four Major Cancers

Colorectal Adenocarcinoma

Non-small cell lung cancer

Endometrial

Melanoma
Copy Number Loss (>2000) as a Potential Resistance Phenotype for Double Non-Responders to Sequential CTLA-4 and PD-L1 Blockade and Loss of Tumor Suppressor Genes on Chromosomes 6q, 10q, and 11q23.3

From: W. Roh et al. (2017) Sci. Trans. Med. 9, eaah3560
Immunotherapy offers hope to some people with hard-to-treat cancers — but it can backfire.

**IMMUNOTHERAPY**

Cancer drugs may speed tumours in some people
The Quest for Precision Immuno-oncology (I/O) Therapy

molecular profiling

clinical data

WGS/WES
Ig/TCR sequencing

SNP assays

RNA Seq

Rx Regimen(s)

adverse events

PFS and OS

• single I/O - dose
• combination - dose
• sequence
• prior Rx

tumor profiling

• mutanome
• neoantigens
• cancer germ-line antigens
• HLA alleles
• clonal/subclonal mutations

immuno-phenotypes

• local (infiltration)
• systemic
• Rx response
• Rx resistance
Characteristics of Immune Cell Infiltrates in 19 Solid Tumor Types
Predictive Biomarkers for Response to Immune Checkpoint Blockade and Other Immunostimulatory Anti-Tumor Therapies

- high negative predictive value
  - reliable identifier of NR cohort
- pre-treatment marker desirable but improved response prediction early in therapy would still be valuable
  - efficacy (R), safety and avoid cost of futile therapy (NR)
- minimally invasive to enable dynamic monitoring
- utility in different cancers
- availability/regulatory approval for all markets in which Rx is sold
Biomarkers for Immunophenotyping

- multiplex panels
- “biomarker positivity”: present, absent or graduated?
- how will reagents, assays and cut-off thresholds be standardized and validated?
- if pre-I/O-Rx (baseline) tissue biopsy is not a robust prediction for R or NR status when and how should post-I/O-Rx profiling be done?
- can immunophenotyping in blood accurately mirror intratumoral events in disseminated metastases with heterogeneous TMEs?
- is there an ‘over-arching’ immunophenotype characteristic for ‘R’ patients that will be valid for diverse I/O-Rx classes with different MOAs?
Need for New Minimally-Invasive Assays for Monitoring Patient Responses to Immunotherapy

- ‘static’ snapshot of immune profile in resected lesions/biopsies versus longitudinal monitoring of dynamic changes with tumor progression/Rx responses

- how far does the immune profile assayed in blood (liquid biopsy) mirror Intratumoral events in anatomically dispersed metastases?
  - immune cell subsets?
  - cytokines?
  - ctDNA?
  - exosomes?
Liquid Biopsy: CTCs and/or ctDNA/RNA
Tumor-Derived Exosomes as a Potential Molecular Profiling Platform to Assess Variation in Immunotherapy Efficacy

Exosomes and Modulation of Immune Functions

An Emerging Component in the Complex Balance Between Immune Activation and Suppression in Tumor-Host Interactions
Putative Roles of Tumor-Derived Exosomes in Cancer Metastasis and Immuno-evasion

Adapted from N. Syn et al. (2016) Trends in Pharmacological Sciences 37, 606
Immune Stimulation by Cancer-Derived Exosomes

- Direct activation of effector T cells by MHC class I and II complexes on vesicle membrane
  - T cell priming required
- Transfer of tumor neoantigens to dendritic cells (DCs) and other antigen-presenting cells (APCs)
- Exosomes stimulation of naïve CD4+ T cells by DC-derived exosomes
- Stimulation of pro-inflammatory macrophage M1 phenotype
Cancer–Derived Exosomes and Suppression of Anti-Tumor Immune Functions

Changes in gene expression; Inhibition of NK cell function; DC and T cell dysfunction; M1→M2 conversion of macrophages

Induction of antigen-specific tolerance
Decoy NKG2D ligands
Apoptosis induction through death receptors
Generation of extracellular adenosine
Cytokine-mediated immunosuppression: induction of Treg cells; promotion of MDSC differentiation

Profiling of Host Immune Cell-Derived and Tumor-Derived Exosomes as Potential Prognostic/Predictive Markers in Immunotherapy
Plasma Tumor-Derived Exosomes

- exosome fraction elevated in cancer patients
- literature reports of up to 150μg protein/ml plasma versus <15μg/ml for normal controls
- preclinical and clinical literature indicating correlation of exosome levels with tumor stage and grade
- emerging evidence in evaluation of response to therapy
  - exosome reduction in long term AML remission plus shift in molecular profile to resemble normal controls
  - reduction in immunosuppressive markers
Blood-based Exosome Profiling

- $10^{12}$ exosomes/ml
- proprietary aptamer oligonucleotide multiplex assay
  - NGS readout of selective binding
- identify cell-of-origin
- quantitative and qualitative disease-associated changes
  - identified by ADAPTamer libraries
  - MDx and Rx target identification
ADAPTamer Library Enrichment Schemes

**Plasma**
- Cancer
- Non-Cancer

- diagnosis and subtyping
- disease progression and Rx efficacy
- immunophenotyping
- responder: non-responder profiling

**Tissue**
- Cancer
- Non-Cancer

- responder: non-responder profiling
- identification of novel druggable resistance targets
- polyligand ADAPTamers and Rx targeting

Tumor-Derived Exosomes as a Potential Molecular Profiling Platform to Assess Variation in Immunotherapy Efficacy

• assessment of $\text{Exo}^{\text{stim}}$ and $\text{Exo}^{\text{supp}}$ ratio in tumor progression and therapeutic efficacy
  – baseline before immunotherapy
  – effect of prior Rx (1L, 2L) on baseline
  – measure dynamic changes in ratio during immunotherapy
  – patterns and ratios in R and NR cohorts and/or adverse events
Validation of Potential Prognostic-Predictive Utility of Exosome Immune Suppression-Activation Ratio Using Banked Samples from Completed Clinical Trials

• plasma
  – high stability due to small radius of curvature
  – 5 years at -80°C
Retrospective Validation of Prognostic-Predictive Utility of Exosome Immune Suppression-Activation Ratio Using Banked Samples from Completed Trials

Tumor-Derived Exosome Immunophenotype

Host-Derived Exosome Immunophenotype

non-responders?

non-responders but extended survival?

Responders?

Durable Clinical Rx Response

suppression

activation

high

low
A Major Repository of Consented Tissue Sections From Clinically Annotated Patients
Selection of Breast Cancer-Specific ADAPTamer Libraries

Baseline

Enriched Libraries
Differential Binding of ADAPTamer Library Discriminates Trastuzumab Responders from Non-responders

Responder

Non-responder
Trastuzumab Non-Responders Exhibit Strong Nuclear Staining for ADAPTamer Library
Identification of Cancer Neoantigens

- Tumor Samples
- ADAPT Exosome Profiling
- Mass Spectroscopy MHC Epitope Recognition Filter

- In Silico MHC Epitope Recognition Filter
- T Cell Recognition-Activation Functional Assays
- Activation/Suppression of Dendritic Cells

- tissue
- blood
Is Widespread Adoption of Immunotherapy Economically Feasible?

- direct Rx cost and futile Rx cost
- indirect care cost of clinical care
- escalating cost of combination regimens (> $200K)
- extravagant cost of cell-based therapies ($500K - $1.5 million)
- high non-responder fraction complicates reaching QALY threshold of $150K in responders
The Evolving Trajectory for Payer Policy for Cancer Therapeutics

- performance – based pricing
- indication – based pricing
- reference – based pricing
Performance-Based Contracts and Pricing: The Inevitable Future Landscape for Immunotherapy?

- Robust identification of responders and non-responders
- Companion diagnostics and labeling requirements
- Performance-based outcomes and premium pricing
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integration of R:NR immunophenotypes into clinical trials and registration dossier

risk sharing