Biomarkers of cancer immunotherapy

Jason Luke, MD, FACP
Assistant Professor of Medicine
Disclosures

• Consultancy:
  – 7 Hills, Actym, Amgen, Array, AstraZeneca, BeneVir, Bristol-Myers Squibb, Castle, CheckMate, Compugen, EMD Serono, Gilead, Janssen, Merck, NewLink, Nimbus, Novartis, Palleon, RefleXion, Syndax, Tempest, WntRx

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  – AbbVie, Array, Boston Biomedical, Bristol-Myers Squibb, Celldex, CheckMate, Corvus, Delcath, Five Prime, Genentech, Immunocore, Incyte, MedImmune, Macrogenics, Novartis, Pharmacyclics, Palleon, Merck, Tesaro, Xencor

• Travel:
  – Amgen, Array, AstraZeneca, BeneVir, Bristol-Myers Squibb, Castle, CheckMate, EMD Serono, Gilead, Janssen, Merck, NewLink, Novartis, RefleXion
PD-L1 dampens the anti-tumor immune response

**IFNγ-mediated up-regulation of tumor PD-L1**


Powderly et al. ASCO 2013
Expression of PDL1 is heterogeneous and varies by biopsy location and antibody stain.

Immunofluorescence shows that stroma and epithelial staining are often concordant and adjacent.

Green = cytokeratin
Blue = nuclei
Red = PD-L1 (SP142)
Given limitations surrounding tumor sampling on biopsy, are there genomic techniques that may assess the tumor microenvironment more robustly?
Tumor mutational burden (SNV or indels) associates with PD1 response and FDA approval of PD1/L1 antibodies
PD-1 antibodies sometimes active in tumors with “zero” PD-L1 expression
TMB and PDL1 improves response selection in NSCLC to nivolumab + ipilimumab

Percent progression-free

log-rank for trend $p = 0.01$

Percent PD-L1 expression

Tumor mutation burden

$62.5\%$ (15/24)  $33.3\%$ (4/12)  $14.3\%$ (3/21)  $7.7\%$ (1/13)

Hellmann et al. Cancer Cell 2018
Total exome mutations correlate with mutations via hybrid capture-based next-generation sequencing in commercial assays.

Based on in silico analysis filtering on 315 genes in FoundationOne comprehensive genomic profile
(Foundation Medicine, Inc, Cambridge, MA, USA)

Mutational load in TCGA skin cutaneous melanoma (SKCM) samples using 315 genes included on the hybrid capture NGS panel is highly correlated with mutations assessed by WES
(Foundation Medicine, Inc, Cambridge, MA, USA)

Peters et al. AACR. 2017
## DNA Repair Defects: Surrogate for TMB?

**Table 2.1** Essential genes of the five major DNA repair mechanisms

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Genes</th>
</tr>
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<tbody>
<tr>
<td><strong>Base excision repair (BER)</strong></td>
<td>DNA glycosylase, APE1, XRCC1, PNKP, Tdp1, APTX, DNA polymerase β, FEN1, DNA polymerase δ or ε, PCNA-RFC, PARP</td>
</tr>
<tr>
<td><strong>Mismatch repair (MMR)</strong></td>
<td>MutSα (MSH2-MSH6), MutSβ (MSH2-MSH3), MutLα (MLH1-PMS2), MutLβ (MLH1-PMS2), MutLγ (MLH1-MLH3), Exo1, PCNA-RFC</td>
</tr>
<tr>
<td><strong>Nucleotide excision repair (NER)</strong></td>
<td>XPC-Rad23B-CEN2, UV-DDB (DDB1-XPE), CSA, CSB, TFIIH, XPB, XPD, XPA, RPA, XPG, ERCC1-XPF, DNA polymerase δ or ε</td>
</tr>
<tr>
<td><strong>Homologous recombination (HR)</strong></td>
<td>Mre11-Rad50-Nbs1, CtIP, RPA, Rad51, Rad52, BRCA1, BRCA2, Exo1, BLM-TopIIIα, GEN1-Yen1, Sli1-Sli4, Mus81/Eme1</td>
</tr>
<tr>
<td><strong>Non-homologous end-joining (NHEJ)</strong></td>
<td>Ku70-Ku80, DNA-PKc, XRCC4-DNA ligase IV, XLF</td>
</tr>
</tbody>
</table>

Dexheimer. DNA Repair of Cancer Stem Cells. 2013.
Working Model: Immunobiology of T cell-inflamed & Non-Inflamed Tumor Microenvironment

### T cell-inflamed

- **CD8**
- **FoxP3**
- **PD-L1**
- **IDO**

### Non-T cell-inflamed

Chemokine profile

PFS and OS in Patients With Melanoma and IFNγ Signature Score Above and Below the Cutoff

Ribas et al. J Clin Oncol 33, 2015 (suppl; abstr 3001)
Genetic Landscape of the T Cell-Inflamed Tumor Microenvironment Across TCGA Solid Tumors

Core Gajewski T cell & IFN-γ linked “T cell-inflamed signature”

CD8A, CCL2, CCL3, CCL4, CXCL9, CXCL10, ICOS, GZMK, HLA-DMA, HLA-DMB, HLA-DOA, HLA-DOB

Non-Synonymous Mutation Frequency Does Not Correlate with T Cell Gene Signature in any Cancers Among TCGA

Relationship of IFN-γ related gene expression & TMB with response to anti–PD1 for HNSCC and gastric cancer in KN012 and KN028.

**Merck Interferon-γ signature**

- CCL5  
- CD27  
- CD274  
- CD276  
- CD8A  
- CMKLR1  
- CXCL9  
- CXCR6  
- HLA.DQA1  
- HLA.DRB1  
- HLA.E  
- IDO1  
- LAG3  
- NKG7  
- PDCD1LG2  
- PSMB10  
- STAT1  
- TIGIT

Seiwert et al. ASCO-SITC. 2016
Utility in combining PDL1 & IFN-γ gene expression?

<table>
<thead>
<tr>
<th>IFNγ mRNA/ PD-L1 IHC status</th>
<th>No. of subjects (no. of events)</th>
<th>Median, months$^a$ (95% CI)</th>
<th>Log rank p value</th>
<th>Adjusted hazard ratio$^b$ (95% CI [p value])</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^+$</td>
<td>43 (16)</td>
<td>22.4 (13.4, NR)</td>
<td>0.002</td>
<td>0.42 (0.22, 0.92) [p = 0.011]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^-$</td>
<td>20 (12)</td>
<td>23.1 (6.5, 24.3)</td>
<td>0.044</td>
<td>0.48 (0.23, 0.98) [p = 0.043]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^+$</td>
<td>43 (18)</td>
<td>9.7 (5.1, NR)</td>
<td>0.086</td>
<td>0.64 (0.35, 1.19) [p = 0.160]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^+$</td>
<td>53 (35)</td>
<td>5.9 (4.1, 10.2)</td>
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<tr>
<td><strong>Progression-Free Survival</strong></td>
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<tr>
<td>IFNγ$^+$/PD-L1$^+$</td>
<td>43 (23)</td>
<td>8.8 (3.1, 14.4)</td>
<td>&lt;0.0001</td>
<td>0.38 (0.21, 0.88) [p = 0.001]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^-$</td>
<td>20 (16)</td>
<td>3.8 (1.3, 7.2)</td>
<td>0.071</td>
<td>0.62 (0.33, 1.15) [p = 0.132]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^+$</td>
<td>43 (34)</td>
<td>2.4 (1.3, 3.5)</td>
<td>0.154</td>
<td>0.88 (0.54, 1.48) [p = 0.629]</td>
</tr>
<tr>
<td>IFNγ$^+$/PD-L1$^-$</td>
<td>53 (46)</td>
<td>1.5 (1.2, 2.6)</td>
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</table>
Could RNA sequencing replace IHC for future patient selection?

LUAD
CD274.rnaseq vs CD274.prot greater
Pearson r=0.75418 p<0.0001
358 samples total; 234 kept
If tumors can be phenotyped at baseline as T cell-inflamed or non-T cell-inflamed, is it possible to develop personalized approaches of IO therapy based on this?
Genes Separate Into Those Strongly Correlated and Less Correlated to PDL1

Strongly correlated

CD163, CCR8, PRDM1, SIGLEC1, CD28, FOXP3, CSF1R, CD33, CD4, TLR7, IL10, HLA–DRA, HLA–DPA1, HLA–DQA1, HLA–DOA, CD69, RSAD2, HLA–DMB, CD3E, CD3D, CCL5, CD247, PDCD1, CD8A, SIRPG, CTLA4, IFNG, TBX21, LAG3, KLRL1, LTA, ICOS, TIGIT, CD3G, ITGAL, CXCL10, CXCL9, CD80, CD86, HAVCR2, ITGB2, CCR1, TNFRSF9, IDO1, STAT1, JAK2, PDCD1LG2, HLA–DPB1, HLA–DRB1, HLA–DMA, HLA–DRB5, HLA–DQB1, KIR2DL4, HLA–DQA2, HLA–DOB, HLA–DQB2, BTLA, LAMP3, CD244, CSF2RA, CD14, IL1B, CD40LG, ITGAX, ITGAM, CD68, ICAM1, MICB, IRF4, STAT4, TNF, CD27, CD72, STAT2, CD40, HLA–B, HLA–C, HLA–A, CXCR3, JAK1, CCR6, LY75, CD79A

Less correlated

IL12B, IRF9, ADAM8, NCR1, TNFSF4, KIR2DL3, KIR2DS4, KIR2DL1, KIR3DL2, KIR3DL1, ISG20, TNFRSF18, IL18, CD93, TMEM173, IL1A, STAT3, FCGR3B, IL6, BATF3, CD70, ENTPD1, TGF81, CD79B, I3RA, TNFRSF4, ADORA2A, LAYN, A4GALT, CX3CL1, TNFSF9, IL23A, IL13, CD19, FCER2, TLR9, CLEC4C, CD22, CD160, RORA, BCL6, GATA3, NT5E, IL12A, MME, CCL20, ICOSLG, XBP1, STAT5B, MST1R, TGF83, TGF82, IL17A, CD276, STAT6, EDNRB, SMAD3, VEGFA, IFNB1, MICA, KIR3DL3, TYK2, TNFRSF14, ST6GAL1, RORC, CEACAM8, ARG2, KRT20, VTCN1, CD24, IFNK, NCAM1, MAGEH1, IL17F, IL5, HMGB1, IFNA1, IFNA13, IFNW1, IFNA21, ARG1, IL4, NDUFA2
Association of PD1 and therapeutically relevant molecules across T cell-inflamed spectrum in melanoma
ADaptiVe Biomarker Trial that InformS Evolution of therapy after nivolumab (ADVISE)

Current selection markers:
CD8, PDL1, LAG3, IDO, CSF1R, FoxP3, GITR, NKp46

Future combination options could be included when appropriate combination safety data + potential IHC assays

Pre-tx biopsy

NSCLC, MEL, RCC, Gastric, SCCHN, Urothelial

Screen (28 day)

Biomarker Defined Treatment Selection

- nivo + relatlimab (LAG3)
- nivo + BMS-986205 (IDO)
- nivo + cabiralizumab (CSF1R)
- nivo + ipilimumab (FOXP3)
- nivo + BMS-986156 (GITR)
- nivo + lirilumab (KIR)
- nivo + SBRT

Treat to PD, Toxicity, or 1 yr

ClinicalTrials.gov Identifier: NCT03335540
BMS CA028-001 / UC IRB17-0731
PI: Jason Luke
Microbiome May Mediate Immunotherapy Efficacy

Zitvogel et al. Nat Rev Microbiol. 2017
PCA analysis sorts significant OTUs by responders and non-responders in metastatic melanoma.

63 OTUs identified

N = 42

Matson et al. Science. 2018
OTUs Associated with Responders and Non-Responders

Matson et al. Science. 2018
Support vector machine model and integration of sequencing methods identifies bacterial species associated response to anti-PD-1

<table>
<thead>
<tr>
<th>Responder</th>
<th>Non-responder</th>
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<tr>
<td><em>Enterococcus faecium</em></td>
<td><em>Ruminococcus obeum</em></td>
</tr>
<tr>
<td><em>Collinsella aerofaciens</em></td>
<td><em>Roseburia intestinalis</em></td>
</tr>
<tr>
<td><em>Bifidobacterium adolescentis</em></td>
<td></td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
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<tr>
<td><em>Veillonella parvula</em></td>
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<tr>
<td><em>Parabacteroides merdae</em></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus sp</em></td>
<td></td>
</tr>
<tr>
<td><em>Bifidobacterium longum</em></td>
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</table>

Matson et al. Science. 2018
Summation PCR score significantly higher in responders and ratio of beneficial:non-beneficial OTUs correlates with RECIST.

Matson et al. Science. 2018
Bacteria associated with anti-PD1 response

Gopalakrishnan et al. Science. 2018
Routy et al. Science. 2018
Matson et al. Science. 2018

Bugs across 3 studies associated with response and previously with “gut health”:
Clostridiales, Ruminococccaeae, Faecalibacterium spp., Akkermansia muciniphila, B. fragilis, Bifidobacteria, Enterococci, Collinsella and Alistipes
Phase II Study Evaluating Gut Microbiota Modulation in Combination with Pembrolizumab in Advanced Melanoma

Merck MISP #53690 - Collaboration with Merck and Evelo

**Primary Endpoint:**
- RECIST response rate

**Secondary Endpoints:**
- PFS, Toxicity
- Microbiome Analysis

**Cohort 1:** PD1 Ab naïve

**Cohort 2:** PD1 Ab refractory/resistant

PI: Jason Luke, MD
Requirements for an effective anti-tumor immune response

The Cancer Immunogram

Conclusions

• PD-L1 IHC alone is of limited value as a predictive biomarker

• Tumor mutational burden or IFN-γ may be better but combos even more so

• T cell-inflamed tumor microenvironment may serve as a model for predicting rational combination immunotherapies
  • Immunotherapy combination regimens should be targeted toward either T cell-inflamed or non-inflamed tumors

• Future may include patient level immune target identification and incorporation of tumor extrinsic factors such as microbiome
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– Team Science Award from the Prostate Cancer Foundation
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@jasonlukemd  🌊  @UCCancerCenter  🎥  ASCO Sun June 3rd – Buddy Guy’s Legends BE THERE!!!