The quest for the identification of the genetic determinants of cancer immune responsiveness
There are three golden rules for the successful treatment of any disease…

…Unfortunately we do not know any of them

Anonymous Stanford Professor
Circa 1982
Anti-PD1/PD-L1 in Breast Cancer

Previously treated TNBC (PD-L1+)

Anti-PD-L1 (MPDL3280A)
N=21

Anti-PD1 (Pembrolizumab; 10mg/kg)
N=27

CR/PR=19%

Emens, L et al., AACR, 2015 (abstract)

Nanda R et al. SABC, 2014 (abstract)
(readapted from Page D, MSKCC, 2015)
“Now this is not the end,
It is not even the beginning of the end.
But, perhaps, it is the end of the beginning”

Winston Churchill
The Lord Mayor's Luncheon, Mansion House
November 10, 1942
“Doctors are men who prescribe medicines of which they know little,
...to cure diseases of which they know less,
...in human beings of whom they know nothing”
• How does tumor rejection occur

• Why does rejection occur
• How does tumor rejection occur

• Why does rejection occur
Lesson learned from vaccination studies

Cytotoxic T cells can co-exist in the host with their target cells

Model: gp100 peptide vaccine ± interleukin-2

Immunologic response

Immunologic Paradox

No clinical response

Lee et al, J. Immunol. 1999
Kammula et al, J Immunol 1999
Nielsen et al, J Immunol 2000
Bittner et al, Nature 2000

Monsurró et al, J Immunol 2000
Wang E et al, Nature Biotech 2000
Bedognetti et al, J Trans Med 2011
Schwartzentruber et al, NEJM 2011
Multidimensionality of tumor/host interactions in the context of T cell aimed immunization

1st dimension = TCR/HLA/peptide interaction
2nd dimension = Localization at tumor site
3rd dimension = Importance of co-stimulation at tumor site

4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor microenvironment

Characterizing intratumoral tumor rejection

Pre-treatment

Post-treatment

FNA

Transcriptome
Imiquimod (TLR-7a)-Basal cell Carcinoma

Pre-treat  TLR-7a Treat

Placebo X 4 days
Placebo X 8 days
Treat X 4 days
Treat X 8 days

Interferon Stimulated Genes
STAT1 1/IRF1
Allograft inflammatory factor 1
IL-15/IL-2/IL15 R b
IL-15 R a/IL-2/IL-4/IL-7/IL-9/IL-15 R g
IL6

CCR5 Ligands
(CCL4/CCL5)
CXCR3 Ligands
(CXCL9
CXCL10)

Immune Effector Genes (IEG)
Granzyme A, B, K
Perforin

Panelli et al. Genome Biol 2007
Adoptive therapy + IL-2 (113 pre-treatment melanoma biopsies)

Cluster 1 (LOW): OR: 38% (16/42)
Cluster 2 (MID): OR: 52% (16/31)
Cluster 3 (HIGH): OR: 65% (26/40)

OR Rate: Cluster 1 < Cluster 2 < Cluster 3

enrich P = 0.03

CXCR3/CXCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2

Bedognetti et al, Br J Can, 2013

113 pre-treatment melanoma biopsies
Ipilimumab (anti-CTLA4)

Pre-treatment

Non Responders  Resp

An immune-active tumor microenvironment favors clinical response to ipilimumab

ORIGINAL ARTICLE
Anti-PDL1 (MPDL3280A) – Metastatic cancers

Higher expression of cytotoxic Th1 T-cell markers in tumor tissue is associated with MPDL3280A activity

Modified from Powderly JD, MD at 2013 ASCO Annual Meeting (left)
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

n=75
The Continuum of Cancer Immunosurveillance: Prognostic, Predictive, and Mechanistic Signatures

Jérôme Galon,1,2,9, Helen K. Angell,1,2,3 Davide Bedognetti,4 and Francesco M. Marincola4,5,*

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Analysis of independent microarray datasets of renal biopsies identifies a robust transcript signature of acute allograft rejection

Pierre Saint-Maurice1, Coline C. Bortkiew1, Wei Zhang1, Alexandre Herbig1, Sergio Kaiser1, Martin Schumpeter1, Grazyna Wilczynska1, Marcel Bigaud1, Jeanne Kelhen1, Eric Rondeau1, Friedrich Rauf1 and Hans-Peter Mertl1

ESOT 2009
The immunologic constant of rejection

Ena Wang1,2, Andrea Worschech1,2,3 and Francesco M. Marincola1,2

Quiescent status:
- Chronic Inflammation

STAT-1/IRF-1/IFN-γ pathway:
- Switch from innate to adaptive immunity
- Acute Inflammation

Activation of CCR5/CXCR3 pathways:
- Polarized Th1 Response

Activation of the Immuno-effector functions
- Tissue-destruction

Chronic inflammation:
- IFN-γ, IL-12

Acute inflammation:
- IL-2, IL-15
- T and NK cells
- Cytotoxic activation

Immune suppression:
- Unknown mechanism
- CXCR3 and CCR5 ligands
- T and NK cell migration

Autoimmunity:
- Ag/B-cell/T-cell interactions

Allograft rejection:
- Ag/B-cell/T-cell interactions

Clearance of intra-cellular pathogen:
- PAP/Ag/B-cell/T-cell interactions

Immune stimulation:
- Immunootherapy

Tumor rejection:
- Ag/B-cell/T-cell interactions
- Pro-inflammatory agents

Inflammatory switch:
- IFN-γ, IL-12

Trends in Immunology
• How does tumor rejection occur

• Why does rejection occur
Factors influencing immune responsiveness (1)

Immune-mediated rejection

- Host's genetics (i.e. IRF-5, CCR-5 polymorphisms)

- Tumor's genetics (Escape - Intrinsic Biology)
  - Science. 2013 Nov 22;342(6161):967-70

- Environment (i.e. Microbiome)
Conclusion (2)

Host’s Genetics (Germ line)
Cancer Specific Genetics (somatic mutations)
Environment/ hidden factors (microbiome?)

NO RESPONSE

Wang, Uccellini, Marincola, Oncoimmunology, 2015
Factors influencing immune responsiveness

- Host's genetics
- Tumor's genetics
- Environment

Immune-mediated rejection
Genetic drivers of immune responsiveness in Breast Cancer (TCGA)

Bedognetti D and Ceccarelli M – in preparation
ICR genes vs immune suppressive genes

1095 Breast Cancer Samples (TCGA RNA-seq data)
Unsupervised Consensus Clustering

CXCR3/CCR5
Chemokines
CXCL9
CXCL10
CCL5

Th1
signaling
IFNG
TBX21
CD8
IL12B
CD8
STAT1
IRF1

Effector
functions
GNLY
PRF1
GZMA
GZMB
GZMH

Immune
regulatory
CD274
CTLA4
FOXP3
IDO1
PDCD1

K=4, Calinski

Immunologic Constant of Rejection
Unsupervised Consensus Clustering
Stage and Intrinsic Subtypes
Stage and Intrinsic Subtypes
Survival Analysis - TCGA

N = 953

p = 0.0222

p = 0.00457

p = 0.00142
Survival Analysis – Validation (GE)

Lance Miller’s integrated dataset, N=1,943
Does the copy number landscape differ among different immune phenotypes?
Copy Number Variation vs Breast Cancer ICR Immune Phenotypes

Copy Number Variation vs ICR groups

GISTIC on RNAseq Data using RNASeq cluster assignment

ICR4  ICR3  ICR2  ICR1

Amplification
Deletion
Sig level
Copy Number Variation vs Breast Cancer ICR Immune Phenotypes

**Copy Number Variation vs ICR groups**

- **ICR4**
- **ICR3**
- **ICR2**
- **ICR1**

**CNV typifying only ICR4 group**

- **CXCL3**
- **CXCL8**
- **CXCL9**
- **CXCL10**
- **CXCL11**
- **CXCL13**

**CXC chemokines**

- **Amp**
- **Del**

ICR4 → ICR1
Does the mutational burden differ among immune phenotypes?
The prevalence of somatic mutations across human cancer types

Anti-CTLA4 (Melanoma)

Snyder et al, NEJM, 2015

Anti-PD1 (Lung Cancer)

Rizvi et al, Science, 2015
Mutational Burden vs Breast Cancer ICR Immune Phenotypes

Non Silent Mutations
(P=0.0016)
Specific mutations

Are specific mutations in driver genes associated with a different immune phenotype?
Driver genes (Chisqr < 0.05)

TP53

MAP3K1 & MAP2K4
5 Driver genes
### MAP3K1

<table>
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<tr>
<th>Frame Shift</th>
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<th>Nonsense Mutation</th>
<th>NonSilent</th>
<th>Silent</th>
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- Basal-like
- HER2-enriched
- Luminal A
- Luminal B
- Normal-like
- Luminal
- All Subtypes

**Mutation Frequency**

- Frame Shift: Y-axis
- In Frame Del: Y-axis
- Missense Mutation: Y-axis
- Nonsense Mutation: Y-axis
- NonSilent: Y-axis
- Silent: Y-axis

**ICR Cluster Assignment**

- ICR1
- ICR2
- ICR3
- ICR4
By using consensus clustering analysis based on RNA-seq data of ICR genes in >1000 breast cancer samples, we defined 4 major immunophenotypes (ICR1, ICR2, ICR3, and ICR4).

Patients bearing the immune-favorable phenotype (ICR4) experienced prolonged survival.

In this exploratory study we observed genetic variables associated with the favorable immune phenotype:

1) Number of non-silent mutations

2) Specific copy number variations (e.g. CXCL cluster amplification)

1) Mutational status of genes related with oncogenic process (TP53, and MAP3K1/MAP2K4)
“Doctors are men who prescribe medicines of which they know *pharmacogenomics*,

...to cure diseases of which they know functional genomics,

...in human beings of whom they know *the whole genome*”

Doha, December 13th-14th 2015

Personalized Medicine!
Acknowledgments

Sidra Medical and Research Center, Doha, Qatar
Davide Bedognetti
Ena Wang
Wouter Hendrickx

Computer Science and Engineering, QCRI, Doha, Qatar
Michele Ceccarelli
Ines Simeone
In conclusion:

• I hope you liked my talk:

• “Before criticizing anybody, one should walk for at least a mile in that person shoes…

• …Therefore, if the person does not take the criticism well…you are a mile away and he has no shoes!”