ESMO ADVANCED COURSE ON LUNG CANCER IN IMMUNOTHERAPY

What every oncologist should know about tumor immunology?

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Netherlands Cancer Institute, Amsterdam
I have provided consultation, attended advisory boards, and/or provided lectures for: **Amgen, AZ/Medimmune, Bayer, BMS, GSK, Ipsen, Merck Serono, MSD, Novartis, Pfizer, Roche/Genentech, Sanofi, Seattle Genetics** for which NKI received honoraria.

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Cancer Immunotherapy

...fighting cancer but ignoring the tumor...

unleashing and harnessing the immune system to combat cancer....
Cancer immunity cycle

1. Release of cancer cell antigens (cancer cell death)
2. Cancer antigen presentation (dendritic cells/APCs)
3. Priming and activation (APCs & T cells)
4. Trafficking of T cells to tumors (CTLs)
5. Infiltration of T cells into tumors (CTLs, endothelial cells)
6. Recognition of cancer cells by T cells (CTLs, cancer cells)
7. Killing of cancer cells (immune and cancer cells)
...similar to the induction of an anti-viral immune response....
Step 1: Cancer cell death: release of cancer cell antigens
Intratumoral cDC1 required for antitumor immunity
Cross-presentation of tumor antigens by cDC1 to CD8 T cells

Sanchez-Paulete et al., Ann Oncol 2017
**Step 2: Presentation of cancer antigens by dendritic cells, priming of T cells**

- **cDC1**: (BatF3+), cross presentation to CD8 T cells
  - CXCL9, CXCL10
  - Activation of CD4 T cells (through IL-12)

- **cDC2**: CD4 T cell stimulation

- **pDC**: type I IFN production

- Cross-talk between DC subsets at draining LN

Noubade et al., Front Immunol 2019
Step 2: Presentation of cancer antigens by dendritic cells, priming of T cells
Step 3: T cell repertoire should be sufficient to allow anti-tumor immunity
Changes in T cell repertoire in individuals followed for 20 years

Leo Tolstoy, all young people’s TCR repertoires are alike; each old individual TCR repertoire is different
Step 3: What are the best tumor antigens?

T-cells with high affinity TCRs are deleted from the repertoire.

T-cells with no affinity for MHC are also deleted.

T-cells with some affinity for MHC-peptide are positively selected.

T-cells with low affinity for self and high affinity for foreign antigens.
Foreigness leads to best tumor antigens: neo-antigens, microbial antigens
Generation of MSI-H tumor by knock-down of MSH2 in MSS tumor

Mandal et al., Science 2019
MSI-H tumors treated by anti-PD-1 results in high T cell infiltrates

Mandal et al., Science 2019
MSI-H tumors with highest TMB have best outcome to immune checkpoint blockade

Schrock et al., Ann Oncol 2019
CTLA4 plays a role during T cell priming
How does CTLA4 blockade lead to anti-tumor immune responses?

Two not mutually exclusive mechanisms have been proposed:

- **Priming of tumor-reactive T cells**
  - Against shared tumor associated antigens
  - Against mutated (neo) antigens

- **Depletion of regulatory FoxP3+ T cells from the tumor microenvironment**
What could tumor-specific cytotoxic T cells detect on human cancer?

1. Self antigens (to which tolerance is incomplete)
   *Shared between patients*

2. ‘Neo-antigens’, epitopes that arise as a consequence of tumor-specific mutations
   *In large part patient-specific, hence generally ignored*
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

- Generate map of tumor-specific mutations (ExomeSeq)
- Determine which mutated genes are expressed (RNASEq)
- Predict epitopes for each mutation/each HLA-allele in silico
- Screen for T cell recognition of mutated epitopes
NKI-002: Partial response upon anti-CTLA4 treatment

Van Rooij et al., J Clin Oncol 2013
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

Resected tumor material

Isolate tumor cells

Identify tumor-specific mutations

Van Rooij et al., J Clin Oncol 2013
Analyzing the neo-antigen-specific T cell repertoire in human cancer?

Resected tumor material

Isolate tumor cells

Isolate tumor-infiltrating T cells

Screen with MHC multimer technology

Identify tumor-specific mutations

Predict potential epitopes

Van Rooij et al., J Clin Oncol 2013
Strong T cell response against an $\text{ATR}_{S>L}$ neo-epitope within the tumor

Van Rooij et al., J Clin Oncol 2013
Increased magnitude of neo-antigen-specific T cell response under anti-CTLA4

Van Rooij et al., J Clin Oncol 2013
Mutational load and outcome to ipilimumab

Survival in Discovery Set

- >100 mutations (N=17)
- ≤100 mutations (N=8)

P=0.04 by log-rank test
Step 4: Trafficking of T cells to the tumor
Step 4: Trafficking of T cells to the tumor
Step 5: Infiltration of T cells into the tumor (TIL)

Diffuse infiltration with CD8+ TILs

Absence of TILs

Hot

Cold

Keck et al., Clin Canc Res 2014
Step 5: Infiltration of T cells into the tumor

Hendry et al., Front Immunol 2015
Tumor-associated vasculature influences the tumor microenvironment

Healthy tissue

Tumor tissue

In tumors: Disorganized network of low-integrity vessels

- Blunt ends with low blood flow
- Collapsed/narrowed vessel lumens
- Vessel leakiness
- Acidity
- Hypoxia

Healthy cell
Cancer cell
Hypoxic cancer cell
Hypoxia shapes tumor microenvironment

Hypoxic tumor environment

HIF-1α

MDSC

^PD-L1
^accumulation

HIF-1α

HIF-2α

MACROPHAGE

^PD-L1
^switch to M2 phenotype

CCL28
CXCL12
HIF-1α

T_reg

^accumulation
^suppressive function

VEGF-A
HIF-1α

CTL

^PD-L1
^TIM3
^CTLA-4
^OX40
^LAG3
^CD137

DC

^PD-L1
^maturation
^differentiation
^migration

\downarrow proliferation
\downarrow differentiation
\downarrow alteration in effector function
Step 6: Recognition of tumor antigens by infiltrated T cells

- TCR
- pMHC expression
Role for Tils in cancer

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer
Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D., Dionysios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D., Marco Massobrio, M.D., Giorgia Regnani, M.D., Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D., Katia Schienger, M.D., Ph.D., Michael N. Lieberman, Ph.D., Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

Objective Measurement and Clinical Significance of TILs in Non-Small Cell Lung Cancer
Kurt A. Schalper, Jason Brown, Daniel Carvajal-Hausdorf, Joseph McLaughlin, Vamsidhar Velcheti, Konstantinos N. Syrigos, Roy S. Herbst, David L. Rimm

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome
Jérôme Galon, et al.
Science 313, 1960 (2006); DOI: 10.1126/science.1129139

Immunotype and Immunohistologic Characteristics of Tumor-Infiltrating Immune Cells Are Associated with Clinical Outcome in Metastatic Melanoma
Gustau Erdag, Joochen T. Schaefer, Mark E. Smolkin, et al.
Unbiased analysis of the intratumoral TCR repertoire: Approach

1. Excise tumor
2. Single-cell sort CD8+ TIL
3. Deep-sequence paired TCRα/β chains
4. Generate retroviral vectors with identified TCRs

Scheper et al., Nat Med 2018
Unbiased analysis of the intratumoral TCR repertoire: Approach

1. Excise tumor
2. Isolate viable tumor material
3. Single-cell sort CD8+ TIL
4. Deep-sequence paired TCRα/β chains
5. Transduce into healthy T cells & test against matched tumor
6. Generate retroviral vectors with identified TCRs

Scheper et al., Nat Med 2018

N = ~20 (V1.0)
N = 100s (V2.0)
Patient 1 (Melanoma): 60% of the intratumoral TCR repertoire is intrinsically tumor reactive (n=15)

Scheper et al., Nat Med 2018
Patient 3 (primary MSS CRC): 0% of the intratumoral TCR repertoire is intrinsically tumor reactive (n=19)

Patient 3
Microsatellite-stable colorectal cancer

N=94 (T cells analysed)

Scheper et al., Nat Med 2018

N=39 (TCRs obtained)
Step 6: Recognition of tumor antigens by infiltrated T cells
Impaired antigen processing and presentation as mechanism of resistance to immunotherapy
13 NSCLC patients: Mutations in genes involved in antigen presentation

- Pre-immunotherapy

- At acquired resistance

Gettinger et al., Cancer Discovery 2017
Step 7: Killing of cancer cells
Cancer immunity cycle

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