CDDF Immunotherapy Technology Forum

IMMUNO BIOMARKERS DEVELOPMENT IN THE PERIPHERAL BLOOD

Wien, December 7-8, 2015

From Cancer Epigenetics to Epigenetic Immunotherapy of Cancer

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University Hospital of Siena, Istituto Toscano Tumori
SIENA, ITALY
EPIGENETICS

Heritable changes in gene expression not based on modifications of the DNA sequence
EPIGENETIC MODIFICATIONS

Histone modifications

DNA methylation

PHARMACOLOGICALLY REVERSIBLE

HDAC inhibitors (HDACi)

DNMTs inhibitors (DNMTi)

MicroRNA gene silencing

Maio et al, unpublished
EPIGENETICS AND CANCER

DNA methylation

Cancer development and progression

GLOBAL genomic DNA HYPMETHYLATION

GENE-SPECIFIC promoter HYPERMETHYLATION
Can methylation of neoplastic cells influence prognosis and response to therapy?
Melanoma as a tool for cancer research

- Tissue samples readily accessible
- Adaptable to tissue culture
- Amenable to testing of novel therapies
Cutaneous Melanoma Prognosis

Highly heterogeneous survival for patients with identical sub-staging
LINE-1 as a surrogate marker for global genomic methylation status

- Non-LTR retrotransposons
- Alu: 10.6%
- SVA: 0.2%
- Others: 6.0%
- LTR retrotransposons: 8.3%
- DNA transposons: 2.8%
- LINE1 accounts for ≈17% of the genomic DNA
- Non-transposable elements (~55%)

Cordaux et al, Nat Rev Genet, 2009
Kaplan-Meier analysis of 42 stage IIIC melanoma patients survival according to \textit{LINE-1} methylation

<table>
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<tr>
<th># events/ # patients</th>
<th>Extent methylation</th>
<th>Median OS (95%CI)</th>
<th>5 year OS (%)</th>
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<td>11/21</td>
<td>&lt;40.46</td>
<td>31.9 (13.1-inf)</td>
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<td>≥40.46</td>
<td>11.5 (9.2-20.6)</td>
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Sigalotti et al, \textit{J Transl Med}, 2011
Specific methylation profiles associate with survival of stage III C melanoma patients

<table>
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<th>group</th>
<th># events/ # patients</th>
<th>Median OS (95% CI)</th>
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<td>LM</td>
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**LINE-1** methylation correlates with the number and level of expressed CTA

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**Graph 1:**
- X-axis: Number of expressed CTA
- Y-axis: % Methylation
- Trend line: $P = 1.1 \times 10^{-5}$, $Rho = -0.641$

**Graph 2:**
- X-axis: MAGE-A3 mol/β-actin mol
- Y-axis: % Methylation
- Trend line: $P = 0.003$, $Rho = -0.456$

*Maio et al., unpublished*
Can epigenetic modulation of neoplastic cells be used to design novel immunotherapeutic approaches in cancer?
These pathways can be **activated** via I-O agents to counteract tumor-mediated inhibition

These pathways can be **blocked** via I-O agents to counteract tumor-mediated inhibition

APC=antigen-presenting cell; CTLA-4=cytotoxic T-lymphocyte antigen-4; LAG-3=lymphocyte activation gene-3; MHC=major histocompatibility complex; PD-1=programmed death-1; PD-L1=PD ligand-1; PD-L2=PD ligand-2; TCR=T-cell receptor.

Immune check-point(s) blockade-based combinations/sequences holding the most promise for future development

• Vaccines
• Cytokines
• Tumor microenvironment modulating agents
• Selected chemotherapeutic agents
• Targeted therapies
• Epigenetic therapies
# Modulation of CTA expression in cancer cells by DNMTi

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<tr>
<th>Histotype</th>
<th>Cell line</th>
<th>Treatment</th>
<th>MAGE-A1</th>
<th>MAGE-A2</th>
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Tumor immunomodulatory activity of DNMTi *in vitro*

**IIF analysis of of SGI-110-treated melanoma cells**

Coral S. et al, CII 2012

**MAGE-A-specific CTL recognition of 5-AZA-CdR-treated melanoma clones**

Sigalotti L. et al, Cancer Res 2004

**gp100-specific CTL recognition of SGI-110-treated melanoma cells**

Fonsatti E. et al, CCR 2007

GIF analysis of SGI-110-treated melanoma cells

Fluorescence intensity

Coral S. et al, CII 2012

MAGE-A-specific CTL recognition of 5-AZA-CdR-treated melanoma clones

IFN (pg/ml)

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Sigalotti L. et al, Cancer Res 2004
Gene expression profiles modulated by DNMTi in syngeneic murine tumor TS/A

Significantly modulated pathways

- innate immune response (19 genes)
- immune response (19 genes)
- transport (16 genes)
- signal transduction (12 genes)
- defense response to bacterium (5 genes)
- response to virus (5 genes)
- antigen processing and presentation (5 genes)
- G-protein coupled receptor protein signaling pathway (6 genes)
- spermatogenesis (8 genes)
Tumor immunomodulatory activity of SGI-110 in vivo

LINE-1 methylation

Relative LINE-1 methylation (%)

Days

NY-ESO-1 methylation

Relative NY-ESO-1 methylation (%)

MAGE-A1 methylation

Relative MAGE-A1 methylation (%)

A. Covre et al., Semin Oncol 2015

Issa JP., Lancet Oncol 2015
Epigenetic Immunomodulation of Cancer cell

CCR Molecular Pathways

Maio M. et al., CCR 2015
Epigenetic immuno-sequencing

COMBOS

Improve host’s immune system activity

HOST

Check-point mAb

Modulate tumor immunogenicity and immune recognition

TUMOR

Epigenetic drugs

Epigenetic drugs modulate tumor immunogenicity and immune recognition, thereby improving the host's immune system activity.
Antitumor activity of SGI-110 + α-CTLA-4 mAb in TS/A (breast) tumors

A. Covre et al., Semin Oncol 2015
IHC Analysis on tumor and normal tissues

TUMOR

MHC class I

CTRL

mAb 9H10

DHA

CD3

CTRL

mAb 9H10

DHA /mAb 9H10

CD4

CD8

NORMAL TISSUES

CD3

large intestine

dermis

liver

Kidney

A. Covre et al. OncoImmunol, 2015
Epigenetic immuno-sequencing: the NIBIT-M4 Study
EUDRACT 2015-001329-17

SGI-110
5 days q21

Ipilimumab
4 x q21

FPFV October 12, 2015

A.M. Di Giacomo et al. Semin Oncol, 2015
COMBINATION THERAPIES

DNMTi

CTA-based vaccines
MODULATION OF CTA EXPRESSION BY 5-AZA-CdR IN OKT3-ACTIVATED PBMC

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<th>5-AZA-CdR</th>
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CTA EXPRESSION BY 5-AZA-CdR IN OKT3-ACTIVATED PBMC

PBMC
N 5655

MAGE

CTRL

5-AZA-CdR

NY-ESO-1

CTRL

5-AZA-CdR
Host immunomodulatory activity of 5-AZA-CdR in vitro

**HLA class I expression**

- CTRL
- OKT3
- OKT3 + DHA

**Mixed Lymphocyte Reaction**

- Responder alone
- Responder + Stimulator OKT3/IL-2 100 UI/ml
- Responder + Stimulator OKT3/IL-2 100 U/ml/DAC
- Responder + PHA 10 ug/ml
Process & Product

Leukapheresis ➔ Re-infusion

DeMethAVax
Ready to use

Quality control

Enrichment of PBMC

Ex-vivo Stimulation
Medical Oncology and Immunotherapy, University Hospital of Siena
If they ask you anything you don’t know, just say it’s due to epigenetics.