TOWARDS USING EPIGENETIC THERAPY TO IMPROVE CANCER IMMUNOTHERAPY

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Disclosure

• In the past 12 months, I received consulting fees from Sectorial Assets Management and from Citi Research (CitiGroup)

• I receive research funds from MedImmune
DNA methylation reprogramming in Cancer

Hoadley et al, Cancer Cell. 2012
Cancer Cells Become Epigenetically Addicted

De Carvalho et al, Cancer Cell. 2012
DNA Demethylating Agents: Current Paradigm

- Cancer Cells have massive DNA methylation reprogramming (Seems to be true in ALL types of Cancers – TCGA)

- Cancer Cells depend on aberrant DNA methylation profile to survive: “Epigenetic Addiction”

- DNA demethylating agents may work by re-activating aberrantly methylated Tumor Suppressor Genes
Inconsistencies in the Current Model

- If DNA demethylating agents are acting mainly by activating epigenetically silenced tumor suppressor genes, then:
  - Why clinical response is usually delayed?
  - Why DNA methylation markers can not predict clinical response?
Gene Expression Profiles after 5-AZA-CdR Treatment

Expression Profiles After 5-AZA-CdR Treatment

Roulois, *Cell.* 2015
RNA/DNA Sensor Systems

Xuetao Cao, *Nature Immunology* 2009

Roulois *et al* *Cell* 2015
Low Dose 5-AZA-CdR Treatment Induce IRF7 Activation

p-value of overlap: 5.09e-15
(Group 4 versus IRF7 target genes)

Roulois et al Cell 2015
RNA/DNA Sensor Systems

Xuetao Cao, *Nature Immunology* 2009

Roulois *et al* *Cell* 2015
MAVS Activation Forms Prion-like Aggregates

Fajian Hou, Cell (2011)
Low Dose 5-AZA-CdR Treatment Induce MAVS Activation

Figure 2

Control (Mock)
shMAVS (Mock)
Control (5-AZA-CdR)
ShMAVS ...
MAVS
MAVS
Aggregates
6hs 1d 3d

Neg Control
Pos Control
5-AZA-CdR

Poly(I:C)
5-AZA-CdR
- + - - -
- - + + +
Pos Control
5-AZA-CdR
- + - - -
- - + + +
SDD-AGE
IB: MAVS
Poly(I:C)
5-AZA-CdR

MAVS Aggregates
5-AZA-CdR Induces Formation of dsRNA

Roulois, Cell. 2015
5-AZA-CdR Induces Expression of Endogenous Retrovirus

Roulois et al., Cell 2015
MDA5/MAVS/IRF7 Knockdowns Abolishes 5-AZA-CdR Effect on Population Doubling Time

Roulois, Cell. 2015
Ex-vivo:
3D, SFM to enrich for CIC

Live CRC Tumor Bank
At Princess Margaret
Cancer Centre

• Whole-exome seq
• CNV
• DNA Methylation Arrays
• Gene Expression Arrays
• mirArrays

PDX (Patient-Derived Xenograft)
Low Dose 5-AZA-CdR Decreases Colorectal CICs

Culure for 4 weeks

Tumor Grow for 4 months

Sample 181 - CIMP<sup>lo</sup> (Control)

\[ p = 1.49^{13} \]

\[ p = 10^{-9} \]

Roulois, *Cell*. 2015
Summary - Viral Mimicry Model

- Reduced Cancer Cell Proliferation
- Target Cancer-Initiating Cells
- Increase antigen presentation
- Expression of neoantigens - HERVs
- Induce Immunogenic Cell Death
- Increase PDL1/PDL2 expression

Roulois et al., Cell 2015
DNA Demethylating Agents: A New Paradigm

- DNA Demethylating agents seems to work mainly by inducing a “Viral Mimicry State”
  - Explain why reliable DNA methylation biomarkers at TSGs have not been found

- Cancer Initiating Cells seems to be more sensitive to the “Viral Mimicry State” induced by DNA Demethylating agents
  - Explain the delayed response observed in patients

- “Viral Mimicry State” will increase tumor cell immunogenicity
  - Opportunity to combine Epigenetic Therapy with Cancer Immunotherapy
Demethylating Agents induce Viral Mimicry in other Cancer Types

Chiappinelli et al., *Cell* 2015
Enhancing Tumor-Cell “Visibility” to the Immune System with the Use of Viral Mimicry — A Model.
Viral Mimicry by Demethylating agents targets colorectal CICs in mice

Inhibition of DNA Methylation Suppresses Intestinal Tumor Organoids by Inducing an Anti-Viral Response

Yoshimasa Saito, Toshiaki Nakaoka, Kasumi Sakai, Toshihide Muramatsu, Kohta Toshimitsu, Masaki Kimura, Takanori Kanai, Toshiro Sato & Hidetsugu Saito
~10% of CRC Patients Have High IRF7 Signature

TCGA COAD patients (RNA-seq)
Intestinal Stem Cell (ISC) Signature can Predict CRC Prognosis

Cell Stem Cell
Article

The Intestinal Stem Cell Signature Identifies Colorectal Cancer Stem Cells and Predicts Disease Relapse

Anna Marlos-Suárez,1 Francisco M. Barriga,1 Peter Jung,1 Mar Iglesias,2 María Virtudes Céspedes,6 David Rossell,2 Marta Sevillano,1 Xavier Hernando-Mombiela,1 Victoria da Silva-Díaz,2 Purificación Muñoz,2 Hana Clevers,6 Elena Sancho,1 Ramón Mangues,4 and Eduard Batlle1,7,*
Effector CD8 T Cell Signature can Predict CRC Prognosis

SCIENCE

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon, Anne Costes, Fatima Sanchez-Cabo, Amos Kirilovsky, Bernhard Mlecnik, Christine Lagorce-Pogès, Marie Tosolini, Matthieu Camus, Anne Berger, Philippe Wind, Franck Zinzindohoué, Patrick Bruneval, Paul-Henri Cugnenc, Zlatko Trajanoski, Wolf-Herman Fridman, Franck Pages
IRF7 and CD8 T_{eff} Signatures are Positively Correlated in CRC

IRF7 and CD8 T_{eff} Signatures are Positively Correlated in CRC
5-AZA-CdR or Poly I:C (RIGI/MDA5 ligand)
Decrease WNT signalling in CRC

TCF/LEF Reporter (GFP)
Proposed Model for Sensitization to Checkpoint Inhibitors

- Low Antigen Presentation/Load
- High CIC Frequency
- Low CD8 Effector T Cell Infiltration
- Low Sensitivity to Checkpoint Inhibitors

- High Antigen Presentation/Load
- Low CIC Frequency
- High CD8 Effector T Cell Infiltration
- High Sensitivity to Checkpoint Inhibitors

~90% of CRC Patients

Epigenetic Therapy

~10% of CRC Patients
METADUR STUDY:
AN OPEN-LABEL, MULTICOHORT, PHASE II BASKET STUDY OF A HYPMETHYLATING AGENT AZACITIDINE AND DURVALUMAB (ANTI-PDL1) IN ADVANCED SOLID TUMORS

Clinical PI: Dr. Lillian Siu
Scientific PI: Dr. Daniel De Carvalho
Site Leaders: Dr. Anna Spreamico (Ovarian) Dr. Aaron Hansen (Colorectal) Dr. Philippe Bedard (Breast)
Study Fellow: Dr. Neda Stjepanovic
Co-Scientific-Investigator: Dr. Trevor Pugh (Genomics)
Program Manager: Kendra Ross

Funds: MedImmune Celgene Ira Schneider Foundation
**METADUR - Study Design**

### Biopsy:
- scRNA-seq
- WES
- DNA Methylation profile
- Immune Phenotyping (FACS)
- PDX/Organoids and CIC frequency

### Blood:
- Global Demethylation (PBMC)
- Immune Phenotyping (PBMC)
- Monitor tumor burden (cfDNA)
Acknowledgements

Daniel De Carvalho, PhD
David Roulois, PhD
Helen LooYau
Ilias Ettayebi
Shu Yi (Roxana) Shen, MsC
Rajat Singhania, PhD
Aline Planello, PhD
Tiago Medina, PhD
Shengrui (Frank) Feng, MSc.
Fabiola Castro, PhD

Catherine O’Brien, MD/PhD
Yadong Wang
Trevor Pugh, PhD
Arnavaz Danesh, PhD

Lillian Siu, MD
Philippe Bedard, MD
Aaron Hansen, MD
Anna Sprefico, MD
Neda Stjepanovic, MD

MedImmune
Celgene

Ira Schneider Foundation
J.P. Bickell Foundation