Circulating biomarkers associated with resistance to nivolumab and ipilimumab based regimens indicate persistent immunosuppression and activation of STAT3 signaling

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Introduction

• Nivolumab (PD-1 blockade) and ipilimumab (CTLA-4 blockade) are front line therapy for metastatic RCC (1)

• Resistance involves many factors including the innate and adaptive immunity, the activation of oncogenic pathways and the tumor microenvironment (TME) including: exhausted T cells, T regulatory cells, myeloid-derived suppressor cells and tumor associated macrophages (TAMs)

• There are currently no established predictive biomarkers to anticipate lack of response to immune checkpoint inhibitors (ICIs) in RCC

In this study we aimed to:

(1) **Humans:** Investigate associations in immune alterations in lack of response to nivolumab/ipilimumab

(2) **Mice:** Assess a novel drug combination to target innate and adaptive immune alterations

Methods: Part 1

RCC patients treated with nivolumab/ipilimumab at City of Hope

Timepoint 1: Baseline
Timepoint 2: Week 12

Flow cytometry
Luminex

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Results: Part 1

A

IL-6

Baseline

W12

Non

responders

Responders

IL-8

Baseline

W12

Non

responders

Responders

IL-1RA

Baseline

W12

Non

responders

Responders

B

CD8+ T cells

Baseline

W12

Non

responders

Responders

Foxp3+ Tregs

Baseline

W12

Non

responders

Responders

C

pSTAT3

Baseline

W12

CD3+ T cells

CD8+ T cells

CD4+ T cells

CD4+ FOXP3+ Tregs

CD33+ myeloid cells

HLA-DR+ myeloid cells

CD33+ HLA-DR- CD14+

myeloid cells

CD33+ HLA-DR- CD15+

myeloid cells

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Results: Part 1

A

IL-6 Concentration (pg/ml)

Non-responders Responders

Baseline W12

B

Baseline W12

CD8+ T cells

CD4+ T cells

CD4+ FOXP3+ Tregs

CD33+ myeloid cells

HLA-DR- myeloid cells

CD33+ HLA-DR+ CD14+ myeloid cells

CD33+ HLA-DR+ CD15+ myeloid cells

C

Baseline W12

pSTAT3

tSNE 1 tSNE 2

CD3+ T cells

CD8+ T cells

CD4+ T cells

CD4+ FOXP3+ Tregs

CD33+ myeloid cells

HLA-DR- myeloid cells

CD33+ HLA-DR+ CD14+ myeloid cells

CD33+ HLA-DR+ CD15+ myeloid cells
Methods: Part 2

Syngeneic mouse model of RCC: RENCA mouse model

- Day 0
- Day 3 & 5
- Day 7, 9, 11, 13, 15

1. Peripheral blood
2. Tumors
3. Tumor draining lymph nodes

Flow cytometry
ELISA
Results

A. CpG-STAT3ASO +/- PD-1 blockade

B. CD8+ T cells in tumors

C. MHC II+ CD80+ in TDLNs

D. M1/M2 ratio in TME

E. Circulating IL-6

PBS IgG
CpG-STAT3ASO
Anti-PD-1
CpG-STAT3ASO + Anti-PD-1

Treatment

Days post engraftment

0 5 10 15 20

Tumor size (mm³)

0 500 1000 1500

% CD8+ CD3+ CD45+

0 20 40 60

Treatment

PBS IgG
CpG-STAT3ASO
Anti-PD-1
CpG-STAT3ASO + Anti-PD-1

MHC II+ CD80+ in TDLNs

Treatment

PBS IgG
CpG-STAT3ASO
Anti-PD-1
CpG-STAT3ASO + Anti-PD-1

M1/M2 ratio in TME

Treatment

PBS IgG
CpG-STAT3ASO
Anti-PD-1
CpG-STAT3ASO + Anti-PD-1

Circulating IL-6

Treatment

PBS IgG
CpG-STAT3ASO
Anti-PD-1
CpG-STAT3ASO + Anti-PD-1

0.0 0.5 1.0 1.5 2.0

0.0 500 1000 1500

0.0 2000 4000 6000

0.0 4000 8000

0 5 10 15 20

Concentration (pg/ml)

Days post engraftment

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Summary

• Non-responders to Nivo/Ipi have increased immunosuppressive Tregs, circulating cytokines IL-6, IL-8, IL-1RA and high levels of phosphorylated STAT3 suggesting immune suppression particularly within innate immune system.

• IL-6, IL-8 and IL-1RA should be investigated further as potential biomarkers for resistance to nivolumab and ipilimumab.

• Finally, our animal studies suggest, combining STAT3 inhibitor with PD-1 blockade could be a way to overcome immune suppression in RCC.
Acknowledgments

**Immuo-Oncology**

Marcin Kortylewski
Wilson Tang
Dongfang Wang
Ewa Karczewska

**Medical Oncology**

Sumanta Pal
Nazli Dizman
Alexander Chehrazi-Raffle
Luis Meza
Zeynep Zengin
JoAnn Hsu

Core support:
Tim Synold
Lucy Brown
David Rose

Chemistry core:
Piotr

DoD KCRP: W81XWH2210402

City of Hope

CDMRP

DUET Biotherapeutics

Alan Horsanger, CEO

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