Therapeutic activity of the combination of anti-CD137, OX40 and B7-H1 on a spontaneous mouse model of hepatocarcinoma

Aizea Morales-Kastresana
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amoralesc@unav.es
No potential conflict of interests
Activating signals: TNFRSF members CD137 and OX40
Activating signals: TNFRSF members CD137 and OX40
Inhibitory signals

CD80  PD-1

Stop signal to T cell activation
Anergy
Exhaustion

“Don’t kill me” signal

T cell

APC/
Tumor

B7-H1
Inhibitory signals

CD80  PD-1

T cell

CD80  PD-1

Stop signal to T cell activation
Anergy
Exhaustion

“Don’t kill me” signal
Transgenic mouse model of spontaneous hepatocarcinoma (c-myc-OVA tg+)

Inducible generation of HCC: Tet-off system

Ney et al, Hepatology 2009
Doxycycline

Breeding

Birth

c-myc-OVA tg+

NO TREATMENT
Median survival 7 weeks

Ney et al. Hepatology 2009
Targets for anti-CD137, anti-OX40 and anti-B7-H1 are expressed on TILs

TILs in 3-week old c-myc OVA tg+ mice
IMMUNOTHERAPY with mAbs + ACT

TREATMENT

Activated OT1+OT2

α-CD137

α-OX40

α-B7-H1

Combo3

Doxycycline

d -21  d 0

BREEDING  BIRTH

c-myc-OVA tg+

d21  d25

mAbs

Rat IgG
42,42 mm²

Combo3
11,12 mm²

ACT + Combo3
No tumor

Week 6

Rat IgG (0/10)
Combo3 (2/10)
ACT + Rat IgG (0/10)
ACT + Combo3 (4/11)

Combo3

Percent survival vs. Days

Alive/total

- Rat IgG: 1/37
- anti-CD137: 0/9
- anti-OX40: 0/9
- anti-B7-H1: 2/17
- Combo3: 10/37

ns

***
Increased T cell infiltrates upon Combo3 treatment
Combo3 efficacy is dependent on CD8$^+$ T cells

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Graph showing percentage survival over days with different conditions:
- Rat IgG: 0/4
- Combo3: 4/5
- Combo3 + anti-CD8: 0/4
- Combo3 + anti-CD4: 3/4

Legend:
- Rat IgG: Dashed grey line
- Combo3: Dashed black line
- Combo3 + anti-CD8: Dashed grey line
- Combo3 + anti-CD4: Dashed black line

alive/total

Statistical significance:
- *: Significant
- ns: Not significant
Effector machinery is enhanced upon Combo3 Tx

Gated on CD8 TILs

Rat IgG

17.8% ± 12.7
462 ± 185

15% ± 4.5
143 ± 23

1.4% ± 0.1
12 ± 1

Combo3

69.4% ± 6
2083 ± 225

63.5% ± 0.4
371 ± 7

3.1% ± 0.6
39 ± 7

GzmB → Perforin → FasL →
C-myc OVA tg+ mice are tolerized against SIINFEKL

**JMJ cell line**

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<thead>
<tr>
<th></th>
<th>WT</th>
<th>RAG1 KO</th>
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<tbody>
<tr>
<td>Tumor area (mm²)</td>
<td>0/24</td>
<td>0/33</td>
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**HCC tumor fragments**

<table>
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<td>Tumor area (mm²)</td>
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**Birth**

**Tumor fragment s.c.**

**CFSE-labeled splenocytes**

**IVK**

**Naive WT**

**Immunized WT**

**C-myc OVA tg+ mice**

**% Specific lysis**

<table>
<thead>
<tr>
<th></th>
<th>Naive WT</th>
<th>Immunized WT</th>
<th>C-myc OVA tg+ mice</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>53.1</td>
<td>76.6</td>
<td>53.8</td>
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<tr>
<td></td>
<td>46.9</td>
<td>23.4</td>
<td>46.2</td>
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**IVK**
SIINFEKL does not drive Ag-specific T cell response
TILs recognize specific unknown peptides presented by JMJ cell line and secrete IFN \( \gamma \)

H-2K\(^b\) and D\(^b\) binding feasible epitopes (Immune Epitope Database)
CONCLUSIONS

-Combo3 prolongs the survival of c-myc OVA tg+ mice and synergizes with adoptive T cell therapy of activated OT-1 and OT-2 T cells, in a CD8+ T lymphocyte dependent fashion

-Combo3 increases CD3+ lymphocyte infiltrates in tumor nodules, but not healthy liver tissue

-CD8+ TILs are more blastic and show higher expression of Combo3 targets, allowing the readministration of Combo3

-Combo3 prevents the tolerization of transferred activated OT-1 T cells against SIINFEKL

-TILs from Combo3 treated mice recognize unknown epitopes on HCC-derived cell lines, but not SIINFEKL
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Thanks for your attention
Questions?

LOOKING FOR POST-DOC!!

amoralesc@unav.es