Investigating the differential response to immunotherapy of orthotopic tumors compared to subcutaneous tumors

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Subcutaneous Renca tumors respond well to Trimab when compared to kidney tumors.

Renca (Ch⁺/Luc⁺)

Trimab
DR5
CD40
CD137

S.C
I.K

Monitor

D0 D11 - 20 ....D100

Percent survival

Days since tumor injection

SC: 86%
IK: 14%

1 experiment representative of 3
No differences in tumor growth rate between SC and IK

- **SC tumors**
  - TrimAb
  - Control

- **IK tumors**
  - TrimAb
  - Control

1 experiment representative of 3
AIM: Determine the reasons behind the differential responses to immunotherapy of tumors in different locations

**Immune related differences:**
cells and molecules of the tumor microenvironment before treatment

**Differences in intrinsic tumor qualities:**
resistance to apoptosis, MHC expression, morphological/structural differences
Cytometry gating for immune cells in tumors

Before treatment (D12 after tumor cell injection)

Gated on viability

- **CherryNeg**
- **TCRb Neg**

- **DCs**
- **B cells**

- **macrophages**
- **neutrophils**

- **CD8**
- **Treg**
No differences in frequency of immune cells in kidney tumors compared to subcutaneous tumors

**Neutrophils**
- TCRβ/Ly6G+)/CD11b+

**B cells**
- TCRβ+/CD19+

**Macrophages**
- TCRβ+/F4/80+/CD11b+

**DCs**
- TCRβ+/CD11c+

**CD4 T cells**
- TCRβ+/CD4+

**CD8 T cells**
- TCRβ+/CD8+

**TReg**
- TCRβ+CD8+/CD25+/FR4high

4 independent experiments pooled
Differences in macrophage profile between SC/IK tumors

Macrophages

% of total cells

0 5 10 15

sc ik

Graphs show the distribution of CD11b and F4/80 in SC and IK tumors.

CD11b\text{int} F4/80\text{hi}

CD11b\text{hi} F4/80\text{low}

Bar graphs depict the percentage of total cells in SC and IK tumors.

3 independent experiments pooled
F4/80\textsuperscript{hi}CD11b\textsuperscript{int} macrophages express FoxP3 and the mannose receptor (CD206).
M2 macrophage markers predominate in kidney tumors

SC tumors

↑

IK tumors

Factors for the M2 switch
→ LIF

Growth factors
→ GM-CSF
→ M-CSF

 Trafficking
→ CCL2, CCL1
→ CX3CL1, CCL6

Activity
→ Arginase
→ IL-10

Mantovani A et al, 2002
CD4⁺ T cell depletion triggers regression of SC tumors but not IK tumors

*Image of a graph showing percent survival over days since tumor injection for SC and IK tumors with and without T reg depletion.*

- **S.C:** SC control 84% (n=13)
- **IK:** IK control 0% (n=10)

**T reg depletion (D11,12,13)**

1 experiment representative of 3
Immune response after CD4+ depletion may be systemic

Depleting anti-CD4 antibody

Tumor monitoring

Survival

D 0  D 11-12-13  ---D100

S.C  I.K  I.K+S.C  S.C+S.C

T reg depletion (D11,12,13)

SC α-CD4

SC+SC α-CD4

SC+IK α-CD4

Percent survival

days

N=14-20

3 independent experiments pooled
Kidney tumor inhibits rejection of subcutaneous tumor

**SC alone**

N=6

SC T Reg D

SC Control

**SC + SC**

N=6

T Reg D right

T Reg left

Control right

Control left

**SC + IK**

N=8

SC+IK TReg D

SC+IK Control

Days since tumor injected

Anti-CD4 days 11, 12 and 13

1 experiment representative of 3
Kidney tumors do not respond as well as subcutaneous tumors when pieces are transplanted under the skin.

- **Renca**
  - S.C: harvested
  - I.K: reinjected SC

- **Days after tumor transplant**
  - 0
  - 15
  - 11 days later

- **Percent survival**
  - sc → sc α-CD4
  - ik → sc α-CD4

- **One experiment (n=5)**
AIM: Determine the reasons for the differential responses to immunotherapy of tumors in different locations

Immune related differences:
cells and molecules of the tumor microenvironment

Differences in intrinsic tumor qualities:
resistance to apoptosis, MHC expression,
morphological/structural differences

-What do the tumors look like before treatment?
SC and IK tumors are same size / weight before treatment

D10 before treatment

IK tumors

SC tumors

1 experiment representative of 3
Phenotype of tumors: Higher level of MHC I in SC tumor and higher level of DR5 and expression of Fas L in IK tumors.
Kidney tumors are more highly vascularized

(5 tumors, 10 fields/tumor)
No difference in tumor vessel permeability

Evans blue IV injections 26 min tumors taken Elution in formamide 24 h

D0

S.C

I.K

Renca Ch⁺ luc⁺ cells

Evans blue (ng/mg tissue)

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<th>Kidney</th>
<th>Subcutaneous</th>
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N = 5 tumors, representative of 3 experiments
Summary

- Subcutaneous tumors eradicated by Trimab or $T_{\text{reg}}$ depletion but kidney tumors are not eradicated.
- M2 macrophage microenvironment in kidney tumors.
- Higher frequency of $\text{F4/80}^{\text{hi}}\text{CD11b}^{\text{int}}\text{FoxP3}^+$ macrophages in kidney tumors.
- Immunosuppression may be systemic.
- More blood vessels and higher MHCI in subcutaneous tumors.
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