New strategies that target tumor microenvironment and generate local and systemic immune protection

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Can we combine local RT, antibodies to tumor, selected chemo, hormones to alter tumor microenvironment that allow immunotherapy to optimize host immune response for tumor regression?

**Major barriers in immunotherapy**

- Difficult to break tolerance
- Poor recruitment to tumor site
- Strong suppressive environment within the tumor site
- Fast growing tumor in mouse model
- Lack of defined antigens and adjuvant for CTL

Can we combine local RT, antibodies to tumor, selected chemo, hormones to alter tumor microenvironment that allow immunotherapy to optimize host immune response for tumor regression?
Improved Targeting of X-rays
Local Ablative RT mediates tumor regression

RT-mediated tumor regression depends on T cells
Traditional low dose and hyper-fractionated RT can not control tumor

Blood 2009, Cancer Res. 2011, JI 2013, JCI in press
Which immune cells controls tumor growth

B16 $\rightarrow$ RT on day 14: 15Gy x day 14, 15, ad 16

CD8$^+$ T cells are required for therapeutic response to RT
Increase of PDL-1 induces resistance and allows relapse
can RT increase cross-priming of tumor Ag by intratumor DCs?

B16-SIY

14 days
20 Gy
3 days
CD11c+ from tumor
2C T cells
Proliferation Assay

Which cytokines?

No RT-DC+ T
RT-DC+ T
No RT-DC only
RT-DC only

0 5000 10000 15000 20000 25000

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cpm
Why is cross-priming increased

• It is known that IFNs can be induced by viral DNA for cross priming of CTL.
• Hypothesis: RT-induce DNA damage leads to excessive DNA fragments, like viral infection, which trigger IFNs that induce DC maturation and cross priming
• RT induces IFN inside tumor tissues
The therapeutic response to RT is dependent on type I IFNs

RT induced tumor regression depends on STING but not MyD88 and TRIF. While anti-Her2/neu uses MyD88.
RT and anti-PD-L1 reduces MDSC through CTL

A

TUMOR

<table>
<thead>
<tr>
<th></th>
<th>RT</th>
<th>α B7-H1 mAb (but not anti-PD-1)</th>
<th>harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>14</td>
<td>17</td>
<td>20</td>
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B

Re-activated CTL kills Ag+ cells including MDSC and tumor

Day 0

0 10 20 30 40

Days after tumor challenge

0 200 400 600 800

Tumor Volume (mm$^3$)
anti-HER2/neu antibody reduces tumor burden: how?

Oncogenic blockade

FcR mediated kill?

NK

TUMOR

TUMOR
Tumor model that depends on oncogenic signals and FcR dependent in immunocompetent host

Are T cells essential for Ab-mediated tumor regression?

TUBO derived from MMTV-Rat neu Tg FcR and NK dep.

Cancer Cell, 2010
Ab-mediated tumor regression is CD8 dependent: wt and Tg mice

How can antibody trigger immune responses?

- Stress protein or DNA → TLRs?
- Increased Cross-priming
- MyD88
- IFN
- cytokines
- ADCC
- NK
- CD8
- TUMOR
- Stress protein or DNA
Type I interferons are induced and necessary during antibody-mediated tumor regression.

A

Ab-responding tumor

Ab-resistant tumor (complete)

**TUBO Ab or adenovirus-IFN**

**WT mice**

0 1 2 3 4 5 6 wk

B

anti-IFNAR blockade in vivo

**Ab can induce IFN for tumor regression but antibody can not induce IFN in Ab-resistant tumor**

**Could exogenous IFN be potent for targeting tumor?**
How to target tumor with IFN: armed Ab with IFN for Ab-resistant tumor

Anti-EGFR | Linker | IFNβ

B16-EGFR 25ug Ab or Ab-IFN
WT mice

Days after tumor inoculation

Tumor Volume (mm³)

Mouse Ab-resistant B16-EGFR tumor (complete)

- anti-EGFR
- anti-EGFR-IFNβ
- hlg

Anti-EGFR-IFNβ is effective for controlling Ab resistant tumor
Anti-EGFR-IFNb could induce anti-tumor CTL responses through increase of cross-priming.
Targeting tumor with Ab-IFNb depend on DC and increase the cross-presentation

Cross-priming: DC from tumor

- Tumor DCs + 2C T cells
  - **no peptides**
  - **SIY peptides**

- CD11c-DTR BMC
  - anti-EGFR-IFNb
  - anti-EGFR-IFNb+DT

Relative tumor volume to Day 14 vs. Days after tumor inoculation
DCs were the major cell type responding to the anti-EGFR-IFNβ treatment.
Antagonizing PDL-1 expression induced by AB-IFNβ achieved tumor-free outcome.
Why is anti-tumor effect by antibody diminished when tumor is further progressed?

- Ab dose is too low
- Ab fails to penetrate inside tumor
- Tumor grows more rapidly
- Increased tumor induced tolerance
- Suppressive cells are increased: which one?
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