Tumor targeting antibodies bridge innate to adaptive immunity

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Tumor targeting antibodies are screened and selected to kill tumor cells

- Tumor targeting antibodies
  - Oncogenic receptors: anti-Her2/neu
  - CD20 (cell lineage)
  - CD47 (do not eat me signal)

Bi-specific antibody: increase efficacy and reduce toxicity
How can anti-HER2/neu antibody reduce tumor burden: complex mechanisms?

Oncogenic blockade vs FcR mediated kill?

We select WT vs Rag-1 KO mice for stronger ADCC but lack T/B cells to test its impact.
Tumor targeting Ab controls in T cell dependent fashion

Are T cells essential for Ab-mediated tumor regression?

How do antibodies trigger immune response?

Anti-EGFR or anti-Her2/neu

Stress protein or DNA → TLRs?

MyD88

MyD88/HMGB-1, Type I IFN, type II IFN, DC, and T cell dependent

Increased Cross-priming

ADCC

TUMOR

CD8

NK

cytokines

IFN

DC

Inflammatory cytokines

Increased Cross-priming
Major Mechanism for Anti-CD20 Antibodies: Is ADCC vs CDC?

ADCC vs CDC: which is more important
The therapeutic effect of anti-CD20 treatment is CD8 T cell dependent.
DC is necessary for anti-CD20 treatment

1.8*10^6 A20 S.C.

100μg of anti-mCD20 (Anti-mCD20) or hIgG (Ctrl) i.p.

CD8+ T cell

Anti-CD20

DC??

Tumor control

CD11c-DTR

D11

DT i.p. every other day

D12

D15

Anti-CD20

hIgG1

hIgG1 + DT

Anti-mCD20

Anti-mCD20 + DT

DC is necessary for anti-CD20 treatment
The therapeutic function of anti-CD20 is macrophage-dependent

1.8*10^6 A20 S.C. 100μg of anti-mCD20

D26 IFN-γ ELISPOT

D11 Clophosome i.p. twice a week

Ctrl Anti-mCD20 Anti-mCD20 + Clophosome

Ctrl Clophosome

How can both DC and macrophages are essential?
Anti-CD20 induced increase of DC cross-presentation is macrophage-dependent

Isolated DCs from A20-HA-bearing mice treated with anti-CD20 +/- MO

Cross priming IFNg MO dep.

Isolated cell
For type I IFN

T cell responses are IFNRA dependent

Anti-CD20
Mφ
IFN-I
DC
CD8+ T cell
Tumor control
CD47 blockade permits rapid phagocytosis of tumor cells:

**Innate checkpoint blockade?**

CD47 is innate checkpoint that prevent phagocytosis

**NSG mice**

![Graph showing luciferase radiance and percent survival](image)

Cell, 2009

ALL model  Blood; 71(4); 1374–84
T cells are essential for anti-CD47-mediated tumor control in syngenic tumor models

**a**

A20 (lymphoma) systemic

Days after tumor inoculation

Tumor volume (mm$^3$)

- RatIg
- anti-CD47

**b**

MC38 (colon cancer)

Days after tumor inoculation

Tumor volume (mm$^3$)

- ratIgG
- anti-mCD47

**c**

local

Days after tumor inoculation

Tumor volume (mm$^3$)

- RatIg
- anti-CD47

**d**

A20 (i.p. anti-CD47)

Days after tumor inoculation

Tumor volume (mm$^3$)

- RatIg
- anti-CD47

**e**

Nude mice

Days after tumor inoculation

Tumor volume (mm$^3$)

- ratIgG
- anti-mCD47

T cells are essential for anti-CD47-mediated tumor control in syngenic tumor models.
But the protection of anti-CD47 Ab is dependent on CD8 T cells

A: CD8 dependent

B: memory response by rechallenge
Therapeutic effect of anti-CD47 depends on type I IFNs and DC responding to IFN for cross priming.

A: IFN production by DC

B: IFNAR signaling

CD11c-cre x IFNAR^{fl/fl} = no IFNAR on DC

Which pathway to produce IFN: MyD88, like anti-Neu?
Neither MyD88 nor Trif, two major pathways for IFN production, is essential for tumor control by anti-CD47

STING signaling is essential for type I IFNs

Sting dependent: tumor control  DC → IFN production  Cross-Primining

c  d  e

**c**

STING signaling is essential for type I IFNs

**STING** signaling is

**essential for type I IFNs**

**CD8 T cells response**

**Sting dependent: tumor control**

**DC → IFN production**

**Cross-Primining**
STING from DC but not macrophages is required for anti-CD47 therapy

Why can DC but not macrophages be essential for innate sensing? Which type of DNA or DNA complex senses cGAS/STING pathway?
Anti-CD47 increases DNA and tumor uptake by both DC and macrophages

CFSE-labeled MC38 tumor cells + BMDC or BMM → Ab → 1 h.

Edu labeled tumor cells were inoculated → Ab → 4 h → cells harvested Gate: CD45+CD11b+:

Increased DNA uptake by anti-CD47
DCs accumulate more cytosolic DNA after CD47 blockade

DCs infiltration

Day 0

Day 14

Tumor infiltrating DCs

PCR

Cytosolic DNA

Whole cell DNA

{ gDNA, mitoDNA }

Cytosolic mtDNA

(Relative copy number)

ex vivo

Cytosolic DNA

Fold Change (relative to Rat Ig)

(DC)

Mono

MΦ

** Rat Ig

Anti-mCD47

Cytosolic mtDNA

(Relative copy number)

Fold Change (relative to Rat Ig)

(DC)

Mono

MΦ

DC

*** Rat Ig

Anti-mCD47

Anti-mCD47(24h)

Anti-mCD47(72h)
Anti-CD47 promotes tumor-derived mtDNA

Host VS Tumor? Genomic DNA VS Mitochondrial DNA?

Anti-CD47 promotes tumor-derived mtDNA.
Tumor mtDNA is required for type I IFN production and cross-priming in DC

A  DDC reduces mtDNA

B:  IFN-β

C

mtDNA depleted
CD47-SIRPα signaling in DCs controls the phagosomal PH and DNA degradation

A: mtDNA reduced in macrophages but not DC

B: pH in macrophage is reduced

C: pH in DC increase
CD47 on tumor cells plays dominate role in evasion and PD-L1 is an adaptive resistance
IFNAR and STING/IFN on host cells are required for CD47/PDL1-mediated evasion

A
- WT:MC38
- WT:MC38-CD47/PDL1\textsuperscript{DKO}
- IFNAR-/-:MC38
- IFNAR-/-:MC38-CD47/PDL1\textsuperscript{DKO}

B
- WT:MC38
- WT:MC38-CD47/PDL1\textsuperscript{DKO}
- STING\textsuperscript{mut}:MC38
- STING\textsuperscript{mut}:MC38-CD47/PDL1\textsuperscript{DKO}

C
- WT:MC38
- WT:MC38-CD47/PDL1\textsuperscript{DKO}
- CD11C-STING-/-:MC38
- CD11C-STING-/-:MC38-CD47/PDL1\textsuperscript{DKO}
Binding RBC is the major barrier for systemic use of anti-CD47. How can we target tumor better?

Tumor cells have both PD-L1 and CD47 while RBC have only CD47. Bi-specific anti-PDL1-SIRPα maintaining binding to tumor cells while greatly reducing binding to RBC.
Co-targeting of CD47 and PDL1 on tumor cells is required for tumor growth inhibition.
Anti-PDL1-SIRPα enhances intratumoral DC innate sensing
Summary

• Therapeutic effect of anti-CD47 depends on CTL.
• DCs but not macrophage are required for anti-CD47 Ab-mediated cross priming
• While macrophages degrade DNA faster than DC
• Anti-CD47 Ab-mediated type I IFN induction or cross priming by host DC depends on cGAS-STING pathway
• DC can better sense cytosol mtDNA through cGAS-STING pathway
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Large tumors have more CTLA+ Treg and resist to anti-CD20 therapy: new strategy for combination

Anti-CTLA4 therapy overcomes anti-CD20 resistance
Pretreatment with chemotherapy induces more “eat me” signal and synergize with anti-PDL1-SIRPα for tumor eradication.
CD8+ T cells are essential for the therapeutic effect of anti-PDL1-SIRPα.
Is Tumor expressed-PDL1 essential for tumor growth or PD-L1 blockade therapy? No and no

PD-L1 from host cells but not tumor is essential for suppression
• Type I IFN is essential for various anti-cancer therapy induced tumor regression: such as RT, anti-Her2/neu, anti-CD47 and some chemo drugs
• IFN has been used in some cancer therapy to suppress tumor cell proliferation
• Can tumor targeting antibodies bring sufficient IFN to tumor tissues for immune responses:?