Journal Club SS17

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In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy

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Introduction

• Surgery – the main treatment option for most solid tumors

• But: residual microtumors & circulating tumor cells (CTCs) → and may also induce promotion of cancer metastasis

• Immunotherapy may kill residual cancer cells
Immune checkpoint therapy – PD-L1

**PD-L1 binds to PD-1 and inhibits T cell killing of tumor cell**

- Tumor cell
- PD-L1
- Antigen
- T cell receptor
- PD-1
- T cell

**Blocking PD-L1 or PD-1 allows T cell killing of tumor cell**

- Tumor cell death
- PD-L1
- Anti-PD-L1
- Anti-PD-1
- PD-1
- T cell

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Platelets

- No nucleus
- Derived from megakaryocytes
- Important in hemostasis
- Involved in thrombosis
- Modulated inflammation
- Platelet transfusion in thrombocytopenia
Emerging role of platelets in cancer

Platelets and the Hallmarks of Cancer

- Sustaining proliferative signals
- Resisting cell death
- Supporting cancer stem cells
- Metastasis and evading immune detection
- Inducing angiogenesis

Platelets at the interface of thrombosis, inflammation, and cancer
Aime T. Franco, Adam Corken, and Jerry Ware, Blood. 2015 Jul 30; 126(5): 582–588.
Prepublished online 2015 Jun 24. doi: 10.1182/blood-2014-08-531582
Platelets as drug carriers

- For increased efficacy
- Longer live span
- Migrate to the surgical wound
- Interact with circulating tumor cells (CTCs)
- Enhances immune response
- Upregulates PD-L1

Platelets: at the nexus of antimicrobial defence
doi:10.1038/nrmicro3269, Published online 16 May 2014
Aim of the study

- Targeting residual as well as circulating tumor cells by binding a PD-L1 antibody to the surface of platelets
Materials & methods

• Cell lines: mouse melanoma B16F10, mouse mammary carcinoma 4Ti (both expressing luciferase and GFP)
• Mice: C57BL/6 and BALB/c (6-10 weeks old)
• Platelet preparation (P-aPDL1): Isolation from whole blood, conjugation of aPDL1 via a maleimide linker, Platelet activation with thrombin
• ELISA for antibody and cytokine detection (aPDL1, IL-1β, TNFα, IL-6, sCD40L)
• TEM
• Fluorescence microscopy and flow cytometry
• In vivo bioluminescence and imaging of tumors and antibody
• Tail bleeding assay
Materials & methods

- *In vivo* experiments

- I. Therapy model of incomplete resection
- II. Therapy model of incomplete resection and metastasis
- III. Therapy model of recurrent triple negative 4T1 tumor
Results – aPDL1 binding to platelets

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Platelets

Platelets + aPDL1

Platelets-SH + maleimide-aPDL1

aPDL1 intensity (FITC)

0.20%

13.07%

93.16%

Dump channel (FL4)

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Results – Efficiency, Stability, Surface proteins

- Chart a: Conjugated aPDL1 (pg/Platelet cell) vs Added aPDL1 (pg/Platelet cell)
- Chart b: Platelet cell number/mL vs Cell viability at T=0h and T=24h
- Chart c: Loading percentage (%) vs Time (h)

Histograms for Platelet and P-aPDL1 Intensity for CD9, CD41, CD61, CD62P, and CD40L
Results – Platelet activation I

Calcein aPDL1

PMP

P

100nm

2μm

10μm
Platelet activation II

b

Activated P-aPDL1

P-aPDL1

PMPs-aPDL1

d

Activated platelets Non-activated platelets

Releasen GP1b (ng/ml)

Time (h)

Activated platelets Non-activated platelets

TIMP (ng/ml)

Time (h)

e

Activated platelets Non-activated platelets

TIMP (ng/ml)

Time (h)
Transwell experiment

(a) Upper compartment

(b) Unactivated P-aPDL1

(b) Activated P-aPDL1

1.0 μm microporous membrane

Platelets

Cancer cells
Pharmacokinetics
I. *In vivo* therapy of incomplete resection
Local and distant inflammation and immune infiltration

6h post injection

14 days post surgery
14 days post surgery
II. Therapy model of incomplete resection and metastasis
B16F10 Nucleus
III. Therapy model of a recurrent triple negative 4T1 tumor
Conclusion

- Residual tumor cells after surgery were greatly reduced by platelets conjugated to aPDL1
  
  - Effective and stable conjugation of platelets to aPDL1
  
  - Activation of platelets and release of aPDL1 in surgical wound
  
  - Reduction of residual tumor cells as well as metastasis