In Vivo Synergistic Effect of Checkpoint Blockade and Radiation Therapy Against Chordomas in a Humanized Mouse Model

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CNS 2018 ANNUAL MEETING
HOUSTON, TEXAS
OCTOBER 6-10, 2018

Introduction

Currently, there are no murine chordoma cell lines nor transgenic mouse models of chordomas, which prevents us from investigating the interaction between murine chordomas and murine immune cells. Thus, to scrutinize immunotherapy (IT) against chordomas, the development of a humanized mouse model of chordomas, where human thymus and CD34+ stem cells as well as human chordomas are co-transplanted to engraft human immune system into mice, is imperative. We aimed to develop this model and investigate synergistic effect between IT and radiation therapy (RT) against chordomas using this model.

Methods

Fifteen 10-12-week-old NSG mice were sub-lethally irradiated and then implanted with human fetal thymic tissue and CD34+ stem cells, whose HLA-type is partially-matched with that of the U-CH1 chordoma cell line. Reconstitution of immune cells in NSG mice was confirmed 8 weeks post-transplantation and then each animal was injected with U-CH1 subcutaneously. Next, they were treated for 4 weeks as follows: A) control (n=3), B) anti-human-PD-1 antibodies (n=4), C) RT + isotype antibodies (n=3, 8Gy x 4), D) anti-human-PD-1 antibodies and RT (n=5). Anti-tumor activities were monitored via tumor size, flow cytometry, qRT-PCR, and immunohistochemistry.

Results

One week after the treatment, on the irradiated side, (D) demonstrated lowest tumor volume, highest number of human PBMCs, highest % of CD8+ human T cells, highest % of CD45RO+CD4+ human T cells, and lowest % of PD-1+CD8+ human T cells in the tumors via flow cytometry, and highest IFN-gamma in the tumors via qRT-PCR, compared to the other groups with statistical significance.

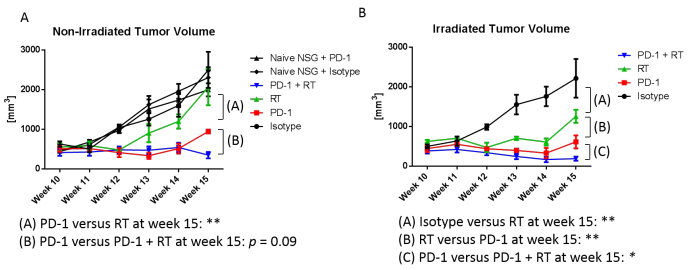
Conclusions

We demonstrated that this humanized mouse model could be a revolutionary platform to investigate IT against rare cancers such as chordomas, where murine equivalents are unavailable. The direct synergistic effect between IT and RT against chordoma was observed, evidenced by lowest tumor volume, highest cytotoxic T cells, and memory T cells.

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the importance of the development of humanized mouse models, 2) Discuss, in small groups, potential limitations of this model such as HLA-partial-mismatch, time-dependent decrease in CD14+ cells and CD19+ cells, relatively small number of regulatory T cells engrafted into NSG mice, and cost and time required to develop humanize mice as well as future directions to overcome these issues, including use of patient-derived xenografts and CD34+ stem cells from the same patients and/or NSG-SGM3 mice, 3) Identify potentially effective treatment options against rare cancers such as chordomas, including immunotherapy (PD-1/PD-L1 blockade and CD47/SIRPa blockade) and combinatorial treatment of immunotherapy and radiation therapy.

Synergistic inhibitory effect of anti-PD-1 antibodies and radiation therapy against chordomas in the BLT NSG humanized mouse model.

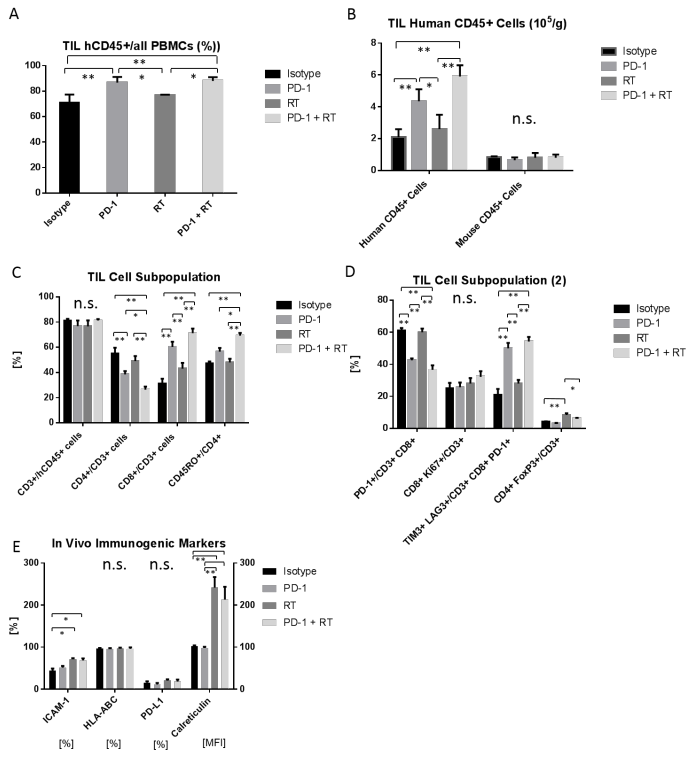


(A) PD-1 versus RT at week 15: **
(B) PD-1 versus PD-1 + RT at week 15: $p = 0.09$

(A) Isotype versus RT at week 15: **
(B) RT versus PD-1 at week 15: **
(C) PD-1 versus PD-1 + RT at week 15: *

(A) on the non-irradiated side (right), the PD-1 + RT group (PD-1 Abs + RT on the contralateral side) harbored the smallest tumor compared to the PD-1 group ($p = 0.09$), the RT-only group (isotype Abs + irradiated on the contralateral side, $p < 0.01$), and the isotype control group ($p < 0.01$). No statistically significant differences were noted amongst naïve NSG mice with isotype-control antibodies, naïve NSG mice with anti-PD-1 antibodies, humanized NSG mice with isotype-control antibodies, and humanized NSG mice with RT on the contralateral side. (B) On the irradiated side (left), the PD-1 + RT group demonstrated lowest tumor volume with statistical significance (versus PD-1 only, $p < 0.05$, versus RT-only, $p < 0.01$, versus isotype, $p < 0.01$)

Flow cytometric analyses of TILs and their immune cell subpopulations.



(A) Tumors in the anti-PD-1 + RT group harbored the highest percentage of human CD45+ lymphocytes as compared with the others ($p < 0.001$). (B) The number of innate Ly5+ cells infiltrating into chordomas were similar across the groups with no statistically significant difference. (C and D) Further analyses on subpopulations of human TILs demonstrated that synergistic increases in CD8+ cells/CD3+ cells (RT versus PD-1 + RT, $p=0.002$, PD-1 versus PD-1 + RT, $p=0.05$) and CD45RO+/CD4+ cells (RT versus PD-1 + RT, $p=0.002$, PD-1 versus PD-1 + RT, $p=0.07$) and decreases in PD-1+ cells/CD3+ CD8+ cells (RT versus PD-1 + RT, $p<0.0001$, PD-1 versus PD-1 + RT, $p=0.26$) by the PD-1 + RT combinatorial therapy were identified.