Efficacy of novel immunogene-combinations for Kras and LKB1 mutant NSCLC in a humanized mouse model

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ABSTRACT

We recently developed an improved humanized mouse by reconstituting a human immune system in NSG mice by transplanting fresh human cord blood derived CD34$^+$ stem cells (Hu-mice). The Hu-mice show functional representation of human T, B, Natural Killer (NK), dendritic cells (DC), myeloid derived suppressor cells (MDSC), and responsiveness to checkpoint blockade. TUSC2 has recently been recognized as a novel immunogene which induces apoptosis in tumor cells and promotes a wide spectrum of tumor-specific innate and adaptive immune responses. We previously reported that TUSC2 delivered systemically by nanovesicles downregulates PD-L1 expression in NSCLC and synergizes with anti-PD1 in inhibiting tumor growth in Kras-mutant syngeneic mouse models through upregulating NK and cytotoxic T cells. In this study, we aimed to evaluate the antitumor efficacy of TUSC2 in combination with standard immunotherapy on highly metastatic Kras and LKB1 mutant human lung cancer in Hu-mice. Hu-mice were challenged with A549 cells (Kras^{mt}/LKB1$^-$) and lung metastasis were treated with TUSC2, nivolumab, or the combination. The results showed a synergistic antitumor effect with the combination. A significantly increased antitumor effect was found when TUSC2 was combined with pembrolizumab in Hu-mice. Pembrolizumab alone significantly reduced tumor burden as compared with an untreated control whereas no antitumor effect was observed in non-Hu-mice implanted with A549 cells. The antitumor effect was correlated with significantly higher levels of CD8$^+$ T and CD8$^+$CD69$^+$ active T and significantly lower levels of MDSC and regulatory T cells in the combination group. A significantly higher percentage of CD56$^+$ NK and CD56$^+$CD59$^+$ active NK cells were found in the TUSC2 alone and combination groups indicating TUSC2 related NK activation. We tested whether TUSC2 enhances efficacy to carboplatin+pembrolizumab in Hu-mice implanted with A549-luc metastatic cells. The results showed that the level of antitumor effect of carboplatin+pembrolizumab was similar to that of TUSC2 alone. But when TUSC2 was combined with carboplatin+pembrolizumab, metastases regression was significantly greater than either TUSC2 alone or carboplatin+pembrolizumab treatments. Significantly fewer or no visible tumor nodules were found in dissected lungs in the TUSC2 combination as compared with other groups. In conclusion, TUSC2 immunogene therapy in combination with pembrolizumab and carboplatin+pembrolizumab showed strong antitumor efficacy in metastatic human NSCLC in a clinically relevant humanized mouse model supporting a clinical trial.

Level of human immune cells in reconstituted humanized mice

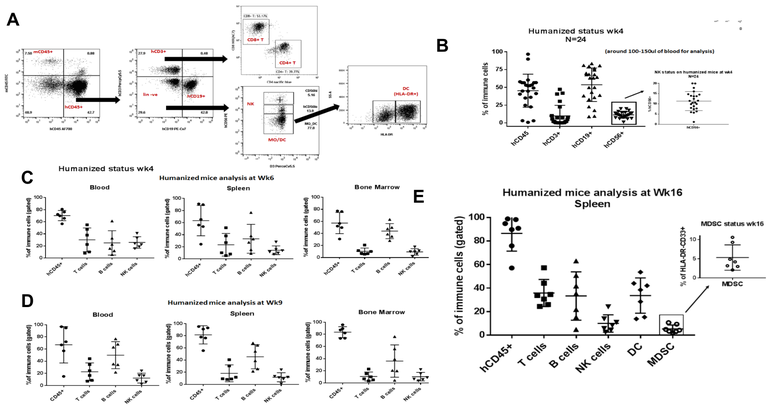

Fig 1. Characterization of humanized mice. A) Gating strategy of multicolor flow cytometry for analysis of human immune cells in mouse organs. B) Analysis of humanization status in PBMC at 4 weeks of post CD34 engraftment. C-D) Analysis of humanized mice at week 6 of post implantation. At this stage, analysis was performed in blood, spleen and bone marrow. E) Myeloid status in humanize mice at week 16.

Antitumor immune effect of pembrolizumab+TUSC2 on lung metastasis in humanized mouse model.

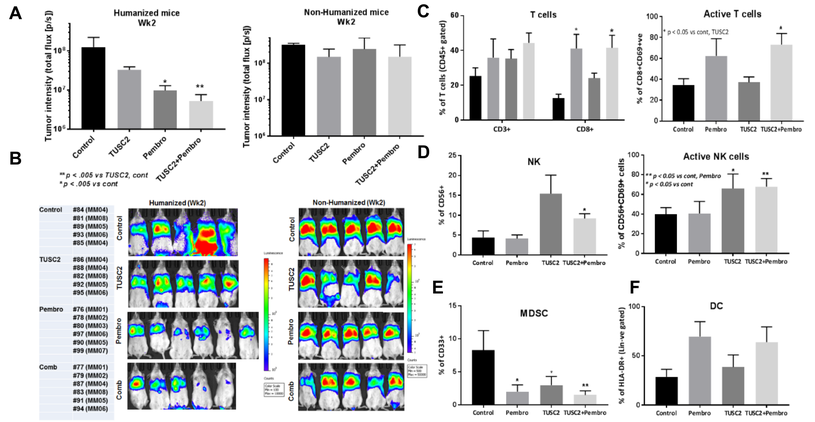

Fig 2. Antitumor effect of Pembrolizumab+TUSC2 on Hu-xenografts. A-B) quantitative analysis(A) and IVIS images(B) Antitumor effect of pembrolizumab in combination with TUSC2, an immunogene, on A549-luc metastases. C-F) Immune response analysis elicited by pembrolizumab +TUSC2 in humanized mice.

Antitumor effect of Carboplatin + pembrolizumab on Kras & LKB mutant A549 human lung metastasis in humanized mouse model

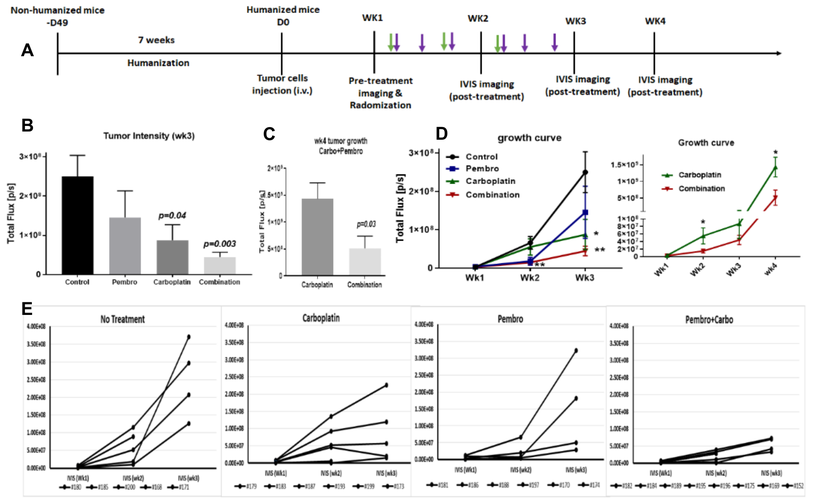

Fig 3. Effect of Carbo+Pembro on humanized mouse model. A) experimental strategy, B-D) quantitative analysis of tumor burden by IVIS imaging (B) wk3, (C) wk4 and (D) tumor growth analysis. E) Comparison of treatment response by analyzing the tumor growth in individual humanized mouse.

Antitumor effect of Carboplatin+Pembrolizumab+TUSC2 on lung metastasis in Humanized mouse model

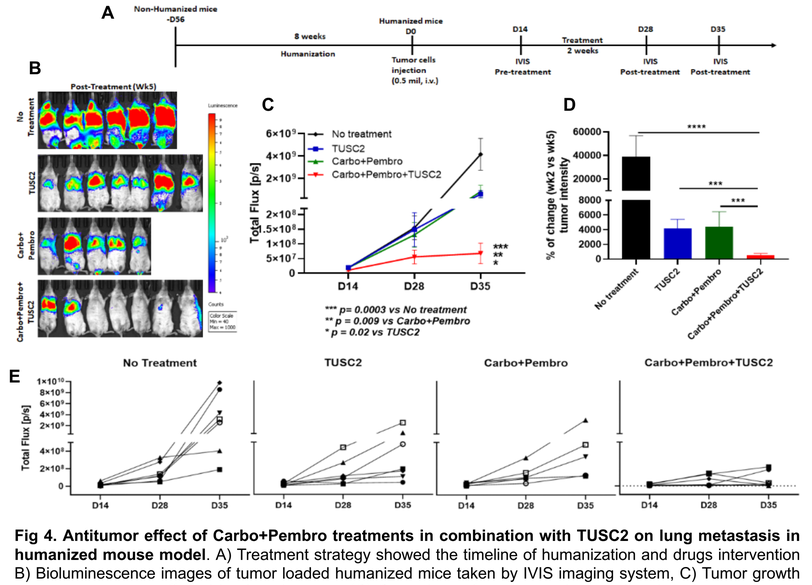

Fig 4. Antitumor effect of Carbo+Pembro treatments in combination with TUSC2 on lung metastasis in humanized mouse model. A) Treatment strategy showed the timeline of humanization and drugs intervention B) Bioluminescence images of tumor loaded humanized mice taken by IVIS imaging system, C) Tumor growth comparison among different treatment groups, (D) Percentage of changes in tumor intensity between pre- and post-treatment, (E) Individual humanized mouse response to treatments determined by assessing the tumor progression.

CONCLUSIONS

- Humanized mice reconstituted from fresh cord blood derived human CD34+ stem cells showed higher level of human immune cells in mouse system which include human T, B, NK, and myeloid cells and persist longer period of time.
- A significantly increased antitumor activity was found when TUSC2, a immunogne therapy, was combined with anti-PD1 therapy (Pembrolizumab) in humanized mouse model.
- TUSC2 and Pembrolizumab combination elicited robust antitumor immune response in humanized mouse system through augmenting activated CD8+T cells, effector NK cells and suppressing regulatory T cells as well as human CD33+ MDSCs.
- Carboplatin and Pembrolizumab combination showed enhanced antitumor activity which is significantly better than single agent treatment in humanized mouse model. This findings resemble in clinic.
- The efficacy of Carboplatin+Pembrolizumab is further significantly improved when TUSC2 was combined. Metastases regression was significantly greater than either TUSC2 alone or carboplatin+pembrolizumab treatments.

References & Disclosures

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