non-small cell lung cancer (NSCLC) in humanized mouse models

Ismail M Meraz[1], Mourad Majidi[1] Meng Feng[1], RuPing Shao[1], Min Jin Ha[2], Elizabeth J Shpall[3], Jack A Roth[1]

Thoracic and Cardiovascular Surgery[1], Biostatistics[2], Stem Cell Transplantation[3] MD Anderson Cancer Center, Houston, TX

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ABSTRACT

TUSC2 is a tumor suppressor gene, whose expression is reduced in almost all NSCLC. Systemic nanovesicle delivery of TUSC2 inhibits cancer cell growth through inhibition of a broad spectrum of kinases and mTOR downregulation as well as stimulation of the immune system through innate activation. We previously reported that TUSC2 downregulates PD-L1 expression in NSCLC and synergizes with anti-PD1 in inhibiting tumor growth in Kras mutant syngeneic mouse models through upregulation of NK and cytotoxic T cells. We developed an improved CD34-derived humanized mouse model (Hu-mice), with faster and higher human immune reconstitution than other available humanized mice, to evaluate immune responses in lung cancer. In this study, we tested whether TUSC2 immunogene therapy would enhance response to standard checkpoint blockade immunotherapy, chemotherapy and targeted therapies in humanized NSG mice implanted with highly metastatic Kras^{mt}/LKB1^{-} A549 cells. A significantly increased antitumor effect was found when TUSC2 was combined with pembrolizumab. Pembrolizumab alone reduced tumor burden as compared with an untreated control, whereas no antitumor effect was observed in non-Hu-mice implanted with A549 cells. The observed antitumor effect correlated with increased levels of CD8+ T and CD8+CD69+ active T, and decreased levels of MDSC and regulatory T cells in the combination group. A significantly higher percentages of CD56+ NK and CD56+CD69+ active NK cells were found in the TUSC2 alone and combination groups indicating TUSC2 related NK activation. Next, we tested whether TUSC2 enhances efficacy to carboplatin + pembrolizumab. The level of antitumor effect of carboplatin + pembrolizumab was similar to that of TUSC2 alone. However, 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. Immune analysis of the triple combination in CMT167 syngeneic mice showed increased infiltration of CD3+ T, CD8+ T, NK cells and significantly less Treg cells into tumor, which was associated with significant tumor inhibition by the treatments. A higher percentage of CD3+CD44+ and CD8+CD44+ memory T cells were found in tumors after carbo+aPD1+TUSC2 treatment, as compared with either Carbo+aPD1 or control groups. The antitumor activity of Carbo+aPD1+TUSC2 was further enhanced when MEKi (Trametinib) was added. Moreover, we also combined TUSC2 with the anti-angiogenic agent, bevacizumab (anti-VEGF) to enhance efficacy in the highly angiogenic 786-O renal cell carcinoma. Synergistic antitumor activity was found with the combination, which was significantly stronger than either single agent. In conclusion, the addition of TUSC2 immunogene therapy with checkpoint blockade, chemotherapy, and targeted therapies showed enhanced antitumor efficacy.

Level of human immune cells in reconstituted humanized mice

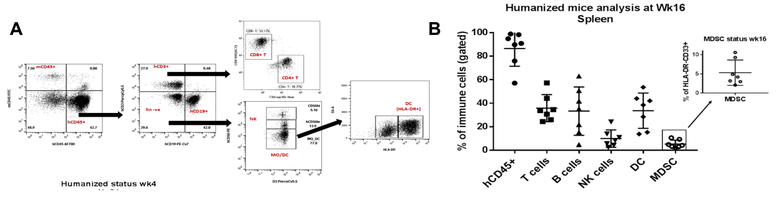

Fig 1. Characterization of humanized mice. A) Gating strategy of multicolor flow cytometry for analysis of human immune cells in mouse organs. B) Analysis of humanization status in spleen at 16 weeks of post CD34 engraftment

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

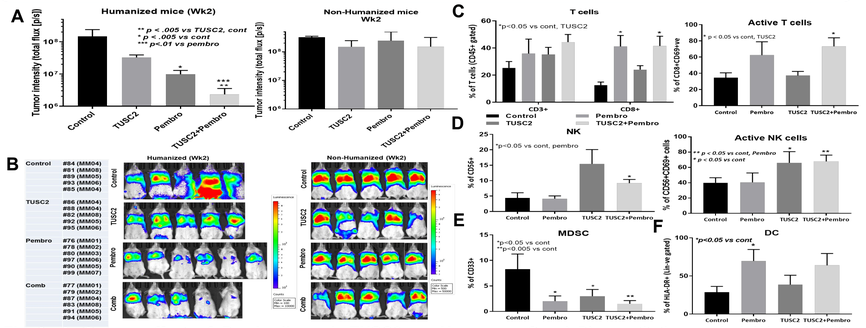

Fig 2. Antitumor effect of Pembrolizumab+TUSC2 on Hu-xenografts. A-B) quantitative analysis(A) and IVIS images(B) Antitumor effect of pembrolizumab in combination with TUSC2, an immunogene, on A549-luc metastases. C-F) Immune response analysis elicited by pembrolizumab +TUSC2 in humanized mice.

Antitumor effect of Carboplatin+Pembrolizumab+TUSC2 on A549 lung metastasis in Humanized mouse model

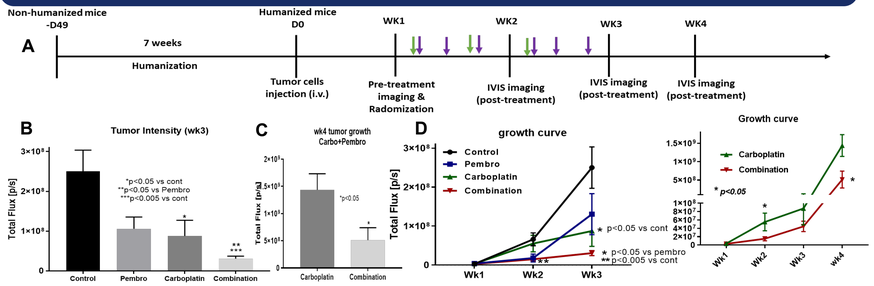

Fig 3. Effect of Carbo+Pembro on humanized mouse model. A) experimental strategy, B-D) quantitative analysis of tumor burden by IVIS imaging (B) wk3, (C) wk4 and (D) tumor growth analysis.

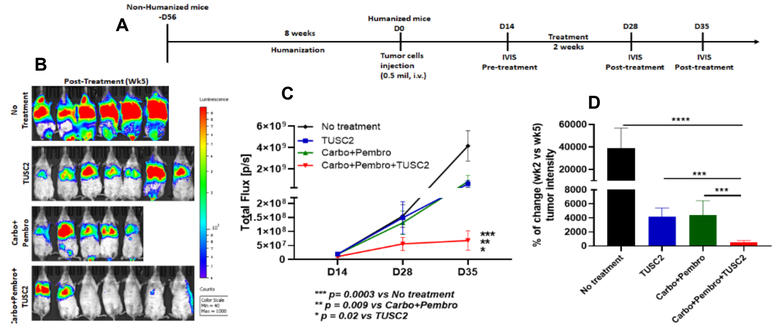

Fig 4. Antitumor effect of Carbo+Pembro treatments in combination with TUSC2 on A549 lung metastasis in humanized mouse model. A) Treatment strategy showed the timeline of humanization and drugs intervention B) Bioluminescence images of tumor loaded humanized mice taken by IVIS imaging system, C) Tumor growth comparison among different treatment groups, (D) Percentage of changes in tumor intensity between pre- and post-treatment.

Effect of Carboplatin+aPD1+TUSC2 on CMT167 tumors in syngeneic mouse model

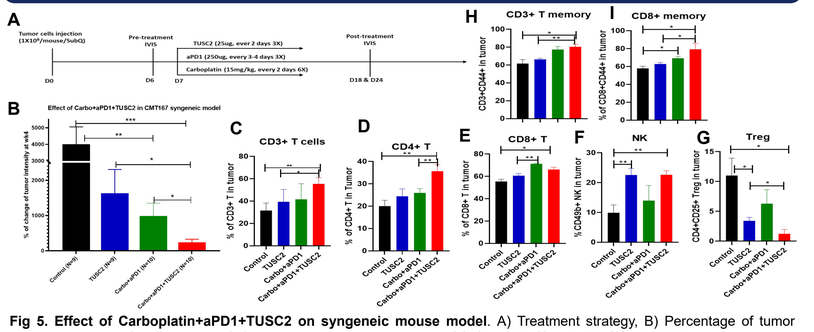

Fig 5. Effect of Carboplatin+aPD1+TUSC2 on syngeneic mouse model. A) Treatment strategy, B) Percentage of tumor intensity changes after treatments, C-I) Immune analysis in tumor by FACS. * p<0.05, ** p<0.005, *** p<0.0005

Antitumor effect of TUSC2 with MEKi & Bevacizumab

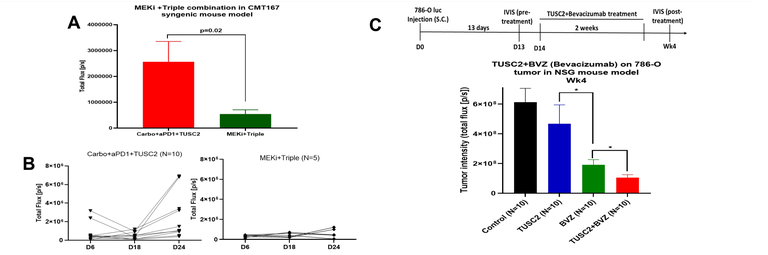

Fig 6. Antitumor effect of TUSC2 with MEKi and Bevacizumab. A) Tumor intensity (Total Flux) at D24 after MEKi (Trametinib) + Triple treatments against CMT167 tumors in syngeneic mouse model. B) Individual mouse response to treatments, C) The tumor intensity after treatments against 786-O Renal Cell Carcinoma model.

CONCLUSIONS

- TUSC2 immunogene therapy showed significant antitumor effect when combined with anti-PD1 therapy (pembrolizumab) in a humanized mouse model which was associated with activated CD8+T cells, effector NK cells and reduced regulatory T cells and human CD33+ MDSCs.
- Carboplatin combined with pembrolizumab showed enhanced antitumor activity which is significantly better than single agent treatment in a humanized mouse model replicating clinical trial results.
- The efficacy of carboplatin+pembrolizumab is significantly increased when TUSC2 was added. Metastases regression was significantly greater than either TUSC2 alone or carboplatin+pembrolizumab treatments.
- The triple combination showed a strong antitumor immune response superior to single agents in a syngeneic mouse model.
- The efficacy of the triple combination was further enhanced with addition of a MEK inhibitor in a syngeneic mouse model
- TUSC2 increased the efficacy of bevacizumab in a human renal cell carcinoma xenograft model.

References & Disclosures

Meraz IM et al, Cancer Immunol Res. 2018 Feb;6(2):163-177.
Meraz IM et al, Cancer Immunol Res. 2019 Aug;7(8):1267-1279

Jack A. Roth is a consultant, stock owner (including pending patent) in Genprex, Inc. All other authors have declared that no competing interests exist.