ACTIVATION OF STAT3 SIGNALING IS ASSOCIATED WITH RESISTANCE TO IMMUNE CHECKPOINT INHIBITORS IN PATIENTS (PTS) WITH METASTATIC RENAL CELL CARCINOMA (mRCC)

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Background Frontline nivolumab plus ipilimumab (N+I) has dramatically improved outcomes in mRCC pts. Nevertheless, only a minority of pts achieve an objective response to therapy. We investigated whether serum cytokine dynamics in pts receiving N+I can elucidate immunotherapy resistance mechanisms. We also evaluated targeted STAT3 inhibition combined with anti-PD-1 in a preclinical model.

Methods Pts who received N+I as first-line treatment of mRCC with baseline and week 12 (+/- 4 weeks) blood samples were identified using an institutional database. Spectral cytometry was used to investigate alterations in immune populations, and the Human Cytokine 30-plex protein assay (Invitrogen) was used to measure circulating cytokines. viSNE projection of peripheral blood analyzed using spectral cytometry was investigated for expression of PD-L1 and STAT3 activation. For studies utilizing syngeneic mouse models of RCC, 6-8 week female Balb/C mice were injected subcutaneously with 500,000 RENCA cells resuspended in a 1:1 ratio of 1x PBS and Matrigel. Mice were treated with either PBS, IgG, CpG-STAT3ASO (a novel oligonucleotide-based TLR9 activator and STAT3 inhibitor), anti-PD-1, or CpG-STAT3ASO plus anti-PD-1. Immune alterations in tumor were assessed via flow cytometric analysis of tumor and in tumor draining lymph nodes. Statistical significance was determined using two-way ANOVA or Wilcoxon signed ranked test with SEM.

Results We evaluated 37 mRCC pts (30:7 M:F) who received N+I, most of whom had clear cell histology (89%) and were IMDC intermediate-risk (76%). Sixteen pts (43%) achieved an objective response, all of which were partial responses. A significant increase in plasma concentrations of cytokines IL-6 (p=0.0046), IL-8 (p=0.0174), IP-10 (p=0.0067), IL-2R (p=0.0174), and IL-1RA (p=0.0079) as well as high pSTAT3 levels were observed in pts who did not respond to N+I. In our syngeneic mouse models, the anti-PD-1 plus CpG-STAT3ASO cohort demonstrated significant tumor growth inhibition vs PBS (p=0.0006), anti-PD-1 (p=0.0285), and CpG-STAT3ASO (p=0.0353) cohorts along with a 4-fold decrease in mean tumor growth when compared to the PBS cohort.

Conclusions Pts unresponsive to N+I exhibited significant increases in cytokines stimulating STAT3 signaling (IL-6, IL-8, and IL-2R) as well as higher levels of active pSTAT3. In addition, our preclinical models indicate that administering anti-PD-1 plus CpG-STAT3ASO leads to significant tumor growth restriction compared to either agent alone. Taken together, these findings suggest that STAT3 activation may be a key mediator of tumor immune evasion and support further exploration of targeting STAT3 in tumor-associated myeloid cells in combination with anti-PD-1 therapy in mRCC.

Ethics Approval The protocol was approved by the institutional scientific review committee, data safety monitoring board, and the institutional review board at the City of Hope Comprehensive Cancer Center. The study conformed with the amended Declaration of Helsinki and the International Conference on Harmonization Guidelines.