

## ANTITUMOR EFFECT OF DA-4507, A NOVEL GCN2 INHIBITOR AS AN IMMUNOTHERAPY IN THE TUMOR MICROENVIRONMENT

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**Background** General control nonderepressible 2 (GCN2) kinase is an integrated stress response (ISR) kinase that responds to amino acid deficiency.<sup>1</sup> Activation of GCN2 in tumor microenvironment (TME), where nutrients are scarce, leads to suppression of immune system and upregulation of translation of proteins for amino acid replenishment.<sup>2</sup> In this study, it was investigated whether immune cells whose function was suppressed in the tumor microenvironment were recovered through selective pharmacological inhibition of GCN2 and whether antitumor effects were exhibited through this. Also, inhibition effects of the tumor intrinsic signaling of GCN2 was evaluated using several cancer cell lines. Taken together, we have shown that inhibition of GCN2 activity could exert anti-tumor efficacy in various ways in the tumor environment.

**Methods** We have discovered a novel small molecule GCN2 inhibitor, DA-4507, that is highly potent and selective *in vitro*. Recovery of effector T cell function and macrophage polarization by DA-4507 were evaluated using mouse primary immune cells under tryptophan-deprived condition. T cell rescue was evaluated using co-culture system with MDSCs accumulated in tumor tissues of syngeneic mouse model. Tumor-intrinsic activities were validated *in vitro* in human leukemia, melanoma, breast cancer, and colorectal cancer cell lines. Also, antitumor effects of DA-4507 were evaluated in the murine syngeneic colorectal, breast cancer and human xenograft leukemia models.

**Results** DA-4507 exhibited high potency (IC<sub>50</sub>=5 nM) and selectivity for GCN2 over other ISR kinases. DA-4507 restored proliferation of cytotoxic T cells and suppressed differentiation of regulatory T cell in low-tryptophan media, where proliferation of T cell was reduced and differentiation of regulatory T cell was increased. DA-4507 also mitigated the suppressive function of MDSCs on T cells and induced macrophage polarization toward inflammatory macrophage phenotype (M1). By blocking the tumor-intrinsic activity, DA-4507 showed anticancer effects in various cancer cell lines in a context-dependent manner. Oral administration of DA-4507 induced a significant reduction of the tumor growth in several murine syngeneic mouse models and a human leukemia xenograft model and decreased ATF4 and phospho-eIF2 $\alpha$  in tumor tissues. We also confirmed that the antitumor effect of DA-4507 is CD8<sup>+</sup> T cell-dependent.

**Conclusions** The activation of GCN2 is known to result in functional suppression of the immune cells and activation of survival pathway of cancer cells in the TME. Our results show that DA-4507, a potent and selective GCN2 inhibitor restores T cell suppression and promotes antitumor activity, demonstrating that GCN2 is a promising therapeutic target for cancer treatment.

### REFERENCES

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<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1343>