Background

General controlled nonderegpressible 2 (GCN2) is a central kinase in the integrated stress response (ISR) that responds to amino acid deprivation. Cancer cells can utilize the ISR for survival, but prolonged or hyperactivation of the ISR reduces proliferation and induces apoptosis. We are developing HC-7366, a First-in-Class, First-in-Human GCN2 modulator that activates GCN2, resulting in anti-tumor activity. HC-7366, currently in a phase I trial (NCT05121948), has demonstrated robust efficacy in multiple pre-clinical solid tumor and AML models.

Myeloid-derived suppressor cells (MDSC) inhibit anti-tumor T cell immunity and promote metastatic spread. Immature myeloid cells are also marked by ISR activation. We hypothesized that HC-7366 treatment could further activate the ISR in MDSCs, leading to cell death or reduced suppressive function and improving anti-tumor immunity. To test this, we used the 4T1 murine breast cancer model, which is characterized by expansion of MDSCs that facilitate lung metastasis.

Methods

4T1 cells were orthotopically transplanted into BALB/c mice. Tumor volume was monitored, and tissues or blood were collected at various timepoints for flow cytometry, IHC, or JESS analysis. Mouse bone marrow derived MDSCs were cultured with T-cells in the presence of HC-7366 in vitro, and anti-proliferative function was evaluated.

Results

HC-7366 treatment showed consistent anti-metastatic efficacy, reducing lung metastases by an average of ~75% across multiple studies. Primary tumors and metastases in treated mice demonstrated GCN2 pathway activation by increases in downstream signaling proteins, including the amino acid biosynthesis proteins ASNS and PSAT1. Anti-tumor efficacy was correlated with significantly decreased Ly6G+ PMN-MDSC frequency in the lungs, spleen, and blood. Additionally, significantly increased expression of the activation markers CD86 and MHCII was observed on PMN-MDSC in both lungs and spleen. Lungs also showed significantly increased T-cell and NK-cell infiltration, activation, and proliferation as measured by increased expression of IL-2, Ki67, T-bet, and Granzyme B. HC-7366 treatment also significantly reduced the S100A8/A9 calcium binding proteins in CD11b+ cells in both metastatic and normal lung tissue, which have been implicated in facilitating MDSC recruitment and proliferation. Reductions in S100A8/A9 were also detectable in PBMCs isolated from peripheral blood and plasma. In vitro suppression assays of bone marrow derived MDSCs co-cultured with T-cells in the presence of HC-7366 showed reduced Arginase 1 expression and T-cell inhibition.

Conclusions

Collectively, these data demonstrate the anti-metastatic efficacy of HC-7366 and its inhibitory effects on MDSCs, outlining its potential as a monotherapy and in combination with other immunotherapeutics to treat MDSC-enriched metastatic cancers.

Ethics Approval

All in vivo experimental procedures were performed in accordance with the NIH Guide for Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee of University of Minnesota. IACUC protocol: 2009A38458