GI-101 Preclinical Data_AACR 2020

6529

GI101, A novel CD80-IgG4-IL2 variant bispecific protein, inhibits tumor growth and induces anti-tumor immune response in multiple preclinical models

Kyoung-Ho Pyo1, Young Jun Koh2, Chun-Bong Synn1, Jae Chan Park2, Jae-Hwan Kim1, Yeongseon Byeon1, Sung Eun Kim1, Ji Min Lee1, Ha Ni Jo1, Woneun Lee3, Do Hee Kim1, Sungwon Park1, Yoo Jeong Song2, Won Jae Lee2, Ji Young Kim2, Hyung Nam Ji2, Sang Su Park2, Kyung Wha Lee2, Young Gyu Cho2, Young Min Oh2, Bo Gie Yang2, Su Youn Nam2, Myoung Ho Jang2, Byoung Chul Cho4

1Yonsei University, Seoul, Korea, Republic of
2Central Research And Development Center, GI innovation, Seoul, Korea, Republic of
3JEUK Institute for Cancer Research, JEUK Co., Ltd, Gumi-City, Korea, Republic of
4Yonsei Cancer Center, Seoul, Korea, Republic of

Introduction: Immune checkpoint blockades (ICBs) have revolutionized cancer treatment and broadened clinical applicability. However, the majority of patients still fail to respond to standard ICBs. To overcome such unmet needs in a clinical study, we designed GI-101, combining the extracellular domain of CD80 serve as a CTLA-4 blockade and an IL-2 variant that preferentially binds the IL-2 receptor β subunit (IL-2Rβ) together. The harmonizing mechanisms of action are projected to translate into improved clinical benefits for this first-in-class immune checkpoint inhibitor fusion protein, even in non-inflamed “cold” tumors.

Methods: Binding affinity of GI101 to IL2Rs, CTLA4, and CD28 was determined by SPR. Immune cell proliferation was analyzed by CFSE assay. In vivo anti-tumor efficacy was tested by single or combination treatment on CT26, MC38 and B16F10 syngeneic tumor models. To elucidate the involvement of GI101 on tumor microenvironment (TME), immune cell population was analyzed by flow cytometry from tumor. Tumor specific T cells (surrogate marker, gp70) were measured by splenocyte proliferation assay and IFN-γ ELISPOT assay. RNA sequencing was performed to elucidate immune mechanism of GI-101.

Results: GI101 highly binds to CTLA-4 (Kd, 2.9 nM) which leads to the reinforcement of endogenous CD80 and CD28 interaction resulting in the activation of T cells. Bivalent IL-2 variant of GI101 triggers both CD8+ T and NK cells proliferation in vitro and in vivo without Tregs proliferation. GI101 has no evidence for toxicity associated with IL-2 activity including vascular leakage syndrome and cytokine storm in non-GLP monkey studies whereas isolated mortality was observed in the anti-PD-1 and anti-CTLA4 combination treatment group. GI101 elicits restoration of immune functions in vitro settings using mouse splenocytes co-cultured with different PDL-1 and CTLA-4 expression level tumor cells. A dose-dependent (3 to 12 mg/kg) inhibition of tumor growth was observed in CT26 syngeneic models without toxicity. Immune profiling of tumor samples also revealed that a robust increment of M1 macrophages, CD8+ central memory T cells (Tcm) and Ki-67+ proliferating T cells but not Tregs in TME (p < 0.05). Tumor specific T cells were strongly proliferated when stimulated with CT26 neoantigens (gp70, RSPWFTTLI and MGPLIVLLL) in splenocyte. IFN-γ+ cells were significantly increased in draining lymph nodes from GI101 treated mice. Furthermore, drastic tumor regression was observed in MC38 tumor-bearing mice treated with GI101 and anti-PD-1 combination.
**Conclusion:** GI101 facilitates the dual function of checkpoint blockade and IL2 activity that enhances the proliferation and activation of T and NK cells. This novel target drug is expected to be interpreted as superior clinical efficacy and safety as indicated even in ‘cold tumor’ models. GI101 is the promising immune-oncology drug to replace the first-generation ICBs by single or combining with other immunotherapies. Our findings provide a rationale for further clinical investigations.

**Keywords:** CD80, IL-2 variant, GI101, Bispecific fusion protein, immunotherapy

---

**Session:** Therapeutic Antibodies 5 / Vaccines (Virtual Poster Session)
**Category:** CLINICAL RESEARCH: Immuno-oncology