**Results**

**Abstract**

Wnt signaling is a fundamental pathway that is dysregulated in oncology. The Wnt signaling modulator DKK1 (expressed in a variety of tumors) is currently being evaluated clinically as a mono- or combination treatment with checkpoint inhibitors. Here we characterize DKN-01, a therapeutic neutralizing monoclonal antibody that binds DKK1 and suppresses Wnt signaling activity in the myeloid compartment, thus providing a rationale for combination treatment with checkpoint inhibitors in the tumor microenvironment by signaling to immune cells. We demonstrate that DKN-01 is a humanized monoclonal therapeutic antibody that binds DKK1 and neutralizes it from 50 to 100 ng/mL. Furthermore, preliminary data suggest that mDKN-01 is targeting a myeloid differentiation inhibitor in nonclinical models. A murine version of DKN-01 (mDKN-01) has efficacy in a syngeneic mouse model of DKK1 tumor promoting activity. DKN-01 was developed in collaboration with CytomX Therapeutics.

**Figure 1: DKN-01 Neutralizes DKK1 in Cell Based Assays**

A) HEK293T cells were stimulated with Wnt3a or combined Wnt3a and Fl1-DKK1 conditioned media. Wnt3a and Fl1-DKK1 combined conditioned media was pre-incubated with increasing amounts of DKN-01 or a human IgG1 control antibody. Phosphorylated LRP5 (p-LRP5) and p-LRP6 levels were measured by western blot (WB) 10 hours after stimulation. Elevated levels of p-LRP5 and p-LRP6 indicates activation of canonical Wnt signaling. Conditioned media from Fl1 vector-transduced cells was used as a control (lane 1 and 2 from the left). B) HEK293 cells with a stably transfected TCF/LEF luciferase reporter were treated with recombinant Wnt3a (200 ng/mL), DKK1 (100 ng/mL), and an increasing titration of DKN-01 or an IgG control antibody for 6 hours. Relative luciferase units (RLU).

**Figure 2: Murine DKN-01 Activity Requires a Functional Immune System**

A) mDKN-01 has Activity in a Mouse Model of DKK1 Tumor Promoting Activity

**Figure 3: Murine DKN-01 Alters the Immune Infiltrate in the Tumor Microenvironment**

Flow cytometry analysis of the B16 tumor microenvironment following murine DKN-01 (mDKN-01) treatment. C57BL/6J mice were inoculated subcutaneously with B16-F0 mouse melanoma cells on Day 0. The following day, bi-weekly intraperitoneal treatment of mDKN-01 (5 mg/kg) or IgG2a control was initiated at 10 mg/kg. Mean tumor volumes and SEM are plotted. *p-val = 0.0003.

**Figure 4: Murine DKN-01 Induces Immune Gene Expression Changes**

**Table 1: DKN-01 Binds Multiple Species of DKK1 with High Affinity**

**Table 2: DKN-01 is Specific for DKK1**

**Conclusions**

- **mDKN-01 has immune modulatory activity and is additive with anti-PD-1 in nonclinical models**
- **Preliminary results from the clinical study of DKN-01 + pembrolizumab demonstrate that the combination was well tolerated and may have activity in patients less likely to respond to anti-PD-1 therapy alone**
- **Clinical study ongoing NCT02013154**