DKN-01, a Therapeutic DKK1 Neutralizing Antibody, Has Immune Modulatory Activity in Nonclinical Tumor Models

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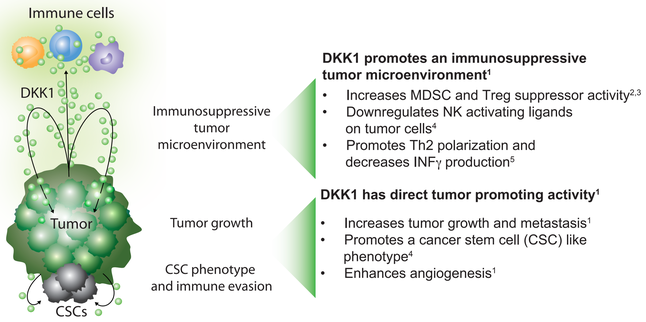
Abstract

Wnt signaling is a fundamental pathway that is dysregulated in oncology. The Wnt signaling modulator DKK1 is expressed in a variety of tumor types and elevated levels frequently correlate with poor survival. DKK1 promotes tumor growth by stimulating proliferation, metastasis, and angiogenesis, and has been implicated in contributing to an immune suppressive tumor microenvironment. DKN-01 is a humanized monoclonal therapeutic antibody that binds DKK1 with high affinity and selectivity. It is currently being evaluated clinically as a monotherapy and in combination in a variety of solid tumors. Here we describe further characterization of the mechanism of action of DKN-01 and demonstrate immune mediated anti-tumor activity in nonclinical models. A murine version of DKN-01 (mDKN-01) has efficacy in a syngeneic melanoma B16 tumor model. However, mDKN-01 is unable to impede B16 tumor growth in NSG immunodeficient mice, indicating that a functioning immune system is required for antibody activity. Furthermore, preliminary data suggest that mDKN-01 is targeting a myeloid derived suppressor cell population in the tumor microenvironment and as such may work in combination with a checkpoint inhibitor. These data support an immune mediated mechanism of action of DKN-01 and provide a rationale for clinical development in combination with immunotherapy agents. The first clinical study evaluating DKN-01 in combination with pembrolizumab has initiated enrollment in patients with relapse/refractory esophagogastric malignancies (NCT02013154).

Introduction

Wnt signaling is a fundamental pathway involved in stem cell maintenance, cell fate decisions, cell proliferation, survival, migration, and polarity determination. Dickkopf-1 (DKK1) is a negative regulator of the canonical Wnt signaling pathway by blocking Wnt interaction with the LRP5/6 coreceptor. In addition, DKK1 has been implicated in activating noncanonical Wnt signaling and PI3K/AKT signaling. DKK1 modulation of these signaling pathways has been linked to promoting tumor growth and metastasis. Overexpression of DKK1 occurs in numerous malignancies and this frequently correlates with a worse clinical outcome. Emerging evidence has implicated DKK1 in promoting an immune suppressive tumor microenvironment by signaling to immune cells. Here we characterize DKN-01, a therapeutic DKK1 neutralizing antibody, and demonstrate that activity in a murine syngeneic tumor model depends on a fully functioning immune system. Our results indicate that murine DKN-01 has immune modulatory activity in the myeloid compartment, thus providing a rationale for combination treatment with checkpoint inhibitors.

Model of DKK1 Tumor Promoting Activity



[1]Kagey and He, BJP, 2017; [2]D'Amico et al., JEM, 2016; [3]Chae et al., Immunology, 2017; [4]Malladi et al., Cell, 2016; [5]Chae et al., Immunity, 2016

Results

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

DKK1 Species	K_D (95% Confidence Interval of fit)
Human	3.3 (1.4-7.5) pM
Murine	7.0 (4.7-11) pM
Rat	8.4 (3.9-23) pM
Rabbit	17 (11-27) pM
Cynomolgus Monkey	14 (8.4-26) pM

The equilibrium dissociation constant (K_D) of DKN-01 was determined by a kinetic exclusion assay (KinExA).

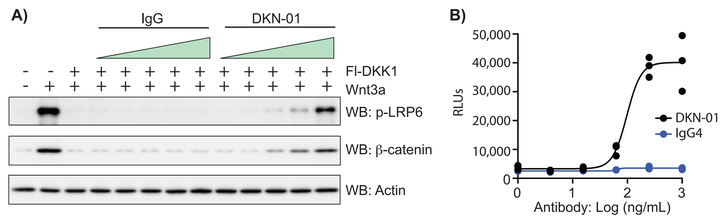
Table 2: DKN-01 is Specific for DKK1

Family Member	K_D
DKK1	3.3 pM
DKK2	> 1 μM
DKK3	> 1 μM
DKK4	> 1 μM

The equilibrium dissociation constant (K_D) of DKN-01 was determined by a kinetic exclusion assay (KinExA) for DKK1, DKK3 and DKK4. The K_D for DKK2 was measured by surface plasmon resonance (Biacore).

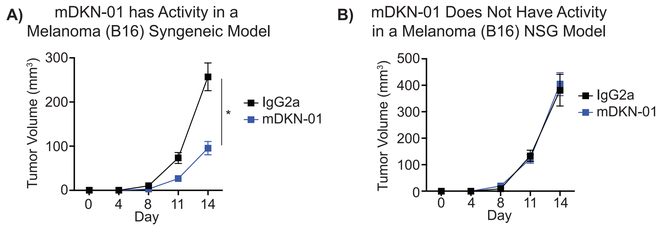
Results

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



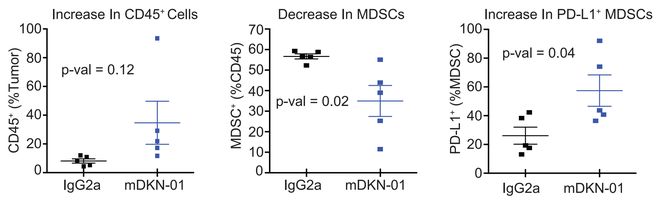
A) HEK293T cells were stimulated with Wnt3a or combined Wnt3a and Fl-DKK1 conditioned media. Wnt3a and Fl-DKK1 combined conditioned media was pre-incubated with increasing amounts of DKN-01 or a human IgG control antibody. Phosphorylated LRP6 (p-LRP6) and β-catenin levels were measured by western blot (WB) 16 hours after stimulation. Elevated levels of p-LRP6 and β-catenin indicates activation of canonical Wnt signaling. Conditioned media from Fl vector transfected cells was used as a control (lane 1 and 2 from the left). **B)** HEK293 cells with a stably integrated 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 IgG4 control antibody for 6 hours. Relative luminometer units (RLU).

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



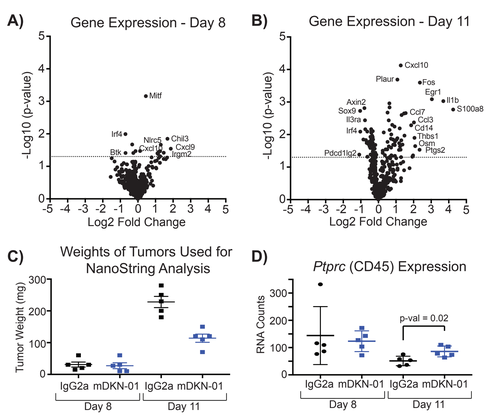
A) Immune competent C57BL/6J mice or **B)** immune incompetent NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice (10 per group) were inoculated subcutaneously with B16-F0 mouse melanoma cells on Day 0. The following day, bi-weekly intraperitoneal treatment of murine DKN-01 (mDKN-01) or IgG2a control was initiated at 10 mg/kg. Mean tumor volumes and SEM are plotted. *p-val = 0.0003.

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



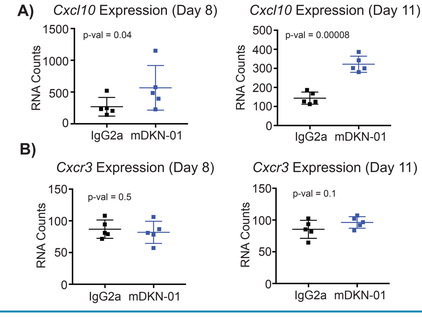
Flow cytometry analysis of the B16 tumor microenvironment following murine DKN-01 (mDKN-01) treatment. C57BL/6J mice were inoculated subcutaneously with B16-F0 mouse melanoma cells on Day 0. The following day, bi-weekly intraperitoneal treatment of mDKN-01 or IgG2a control was initiated at 10 mg/kg. Tumors were isolated on Day 11 (5 per group) and analyzed by flow cytometry for the presence of CD45, myeloid derived suppressor cells (MDSCs) identified as CD11b⁺ and GR-1⁺, and PD-L1⁺ MDSCs. Mean and SEM are shown.

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



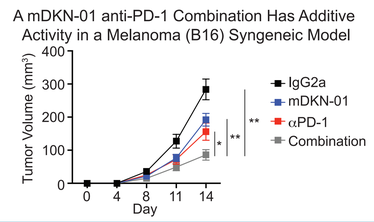
A and B) Volcano plots of NanoString gene expression data from the B16 tumor microenvironment following murine DKN-01 (mDKN-01) treatment. C57BL/6J mice were inoculated subcutaneously with B16-F0 mouse melanoma cells on Day 0. The following day, bi-weekly intraperitoneal treatment of mDKN-01 or IgG2a control antibody was initiated at 10 mg/kg. Tumors (5 per group) were isolated on Day 8 and 11, and purified RNA was analyzed by NanoString using the PanCancer Immune Profiling panel. Data was normalized and differential expression values relative to IgG2a control treatment were calculated with the NanoString nCounter Advanced Analysis Plugin. **C)** Weight of isolated tumors. Mean and SEM are shown **D)** Expression of *Ptprc* (CD45). Mean and standard deviation are shown.

Figure 5: Murine DKN-01 Induces *Cxcl10* But Not *Cxcr3* Expression



A) Murine DKN-01 (mDKN-01) treatment induces expression of *Cxcl10*. NanoString expression data from B16 isolated tumors at Day 8 or 11 following bi-weekly treatment with mDKN-01 or IgG2a (10 mg/kg). RNA counts were normalized with the NanoString nCounter Advanced Analysis Plugin. Mean and standard deviations are shown. **B)** mDKN-01 does not induce expression of the Cxcl10 receptor, *Cxcr3*. Samples were treated and analyzed as described for A.

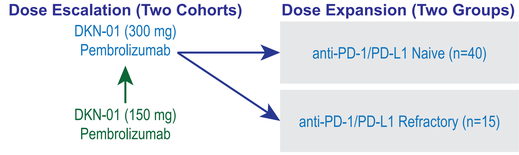
Figure 6: Murine DKN-01 Has Additive Activity With an anti-PD-1 Antibody



C57BL/6J mice (15 per group) were inoculated subcutaneously with B16-F0 mouse melanoma cells on Day 0. The following day, bi-weekly treatment with mDKN-01 (10 mg/kg), bi-weekly treatment with an anti-PD-1 antibody (250 ug/mouse), or combination treatment was initiated. The control animals were treated with rat IgG2a (250 ug/mouse). Mean tumor volumes are plotted. Error bars represent SEM. *p-value <0.001, **p-value <0.0001.

Preliminary Results of Clinical Study Evaluating DKN-01 with KEYTRUDA® (pembrolizumab) in Patients with Advanced Gastroesophageal Adenocarcinoma (GEA)

- DKN-01 administered on Days 1 and 15 of each 21-day cycle, pembrolizumab (200mg) on Day 1 of each 21-day cycle



DOSE ESCALATION SAFETY: Combination was well tolerated with no DLTs or treatment-related SAEs. To date, all treatment related treatment emergent adverse events were Grade 1 (n=4: one event each of hoarseness, flushing, fatigue and diarrhea).

DOSE ESCALATION PRELIMINARY EFFICACY

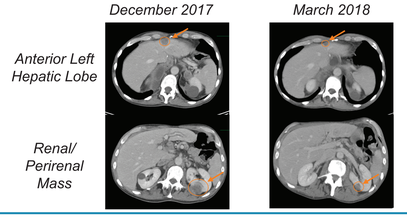
	Prior TX	Medical History	MSI	TMB	PD-L1	BOR	Status
DKN-01 300 mg		**53 M w/GEJ, s/p FOLFOX/trastuzumab, FOLFIRI**	**MSS**	**N/D**	**neg**	**Partial Response (-66%)**	**Cycle 6**
		59 M w/GEJ s/p FOLFOX	MSS	I	neg	Stable Disease (+7%)	Cycle 5
	anti-PD1 PD-L1 naive	74 M w/GC s/p ECF, ramucirumab/paclitaxel	MSS	L	neg	Stable Disease (non-measurable at baseline)	Cycle 4
		61 M w/GEJ s/p FOLFOX, ramucirumab/paclitaxel, irinotecan	MSS	N/D	N/D	Stable Disease (+3%)	Off Study - Cycle 3
		63 M w/ EC s/p FOLFOX, XELOX	MSS	L	pos	Not Evaluable	Off Study (C1 Death - unrelated)
	anti-PD1 refractory	62 M w/GEJ s/p anti-PD1 (PD), ramucirumab/paclitaxel	N/D	N/D	N/D	Stable Disease (+10%)	Cycle 3
DKN-01 150 mg	anti-PD1 PD-L1 naive	67 M w/EC, s/p FOLFOX, ramucirumab/paclitaxel, irinotecan	N/D	N/D	N/D	Progressive Disease (+26%)	Off Study
	anti-PD-L1 refractory	69 F w/GC, s/p FOLFOX, anti-PD-L1 for 2 years with PD	MSS	I	neg	Stable Disease (-10%)	Cycle 6

MSI = Microsatellite Instability.
MSS = Microsatellite Stable.
TMB = Tumor Mutational Burden.
I = Intermediate.
L = Low.
N/D = Not Done/Not Available.

CASE STUDY

Patient with immune resistant phenotype (KRAS amplified, MSS, and PD-L1 negative) who prior to study entry was experiencing rapid disease progression

Confirmed Partial Response (~66% reduction after 5 cycles). Patient continues on study.



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 demonstrated 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

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