Therapeutic Targeting of the Wnt Signaling Antagonist DKK1 with a Humanized Monoclonal Antibody in Oncology Indications

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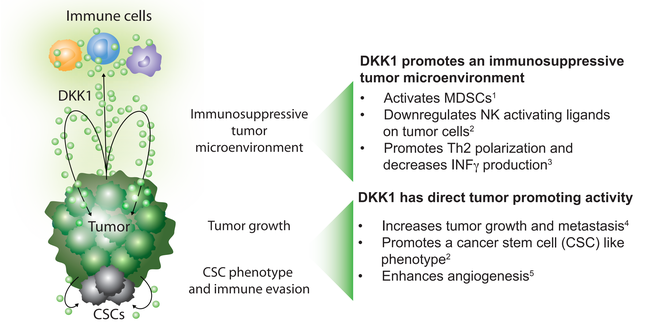
Abstract

Wnt signaling is a fundamental pathway that is dysregulated in oncology. The Wnt antagonist DKK1 is expressed in a variety of tumor types which frequently correlates with a poor prognosis, including overall survival. DKK1 has known oncogenic activity by stimulating proliferation, metastasis, and angiogenesis, and recently been implicated in contributing to an immunosuppressive tumor microenvironment. The neutralization of DKK1 is hypothesized to have efficacy from both a direct anti-tumor effect and through an immune stimulated response. Here we describe the characterization of DKN-01, a humanized monoclonal therapeutic antibody to DKK1. DKN-01 binds DKK1 with high affinity and selectivity, disrupts the interaction of DKK1 with the LRP6 co-receptor, and neutralizes DKK1 activity in a cell based assay. In vivo, DKN-01 has efficacy both as a monotherapy and in combination with chemotherapies in a non-small cell lung (NSCLC) cancer A549 xenograft model. Results suggest that DKN-01 has an antiangiogenic effect and may stimulate a NK cell mediated antitumor response. Clinically, DKN-01 is being evaluated in relapsed/refractory esophageal cancer patients in combination with paclitaxel, and preliminary results demonstrate promising activity. Archival patient tumor samples are currently being analyzed genetically and by IHC for DKK1 and β-catenin staining for biomarker identification. Taken together, our results suggest that DKN-01, a novel therapeutic, has clinical efficacy by disrupting Wnt signaling, which results in a direct anti-tumor effect and stimulates a pro-inflammatory tumor response.

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. Recent studies have indicated that DKK1 is overexpressed in numerous malignancies and this can correlate with a worse clinical outcome. Preclinically, DKK1 stimulates tumor growth, angiogenesis, proliferation and cell migration. DKK1 also signals to immune cells resulting in an immunosuppressive tumor microenvironment. We have hypothesized that DKN-01 has efficacy by disrupting DKK1 signaling to both tumor and immune cells.

Figure 1: Model of DKK1 Tumor Promoting Activity



[1]D'Amico et al., JEM, 2016; [2]Malladi et al., Cell, 2016; [3]Chae et al., Immunity, 2016; [4]Thudi et al., Prostate, 2011; [5]Smadja et al., Arterioscler Thromb Vac Biol, 2010

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).

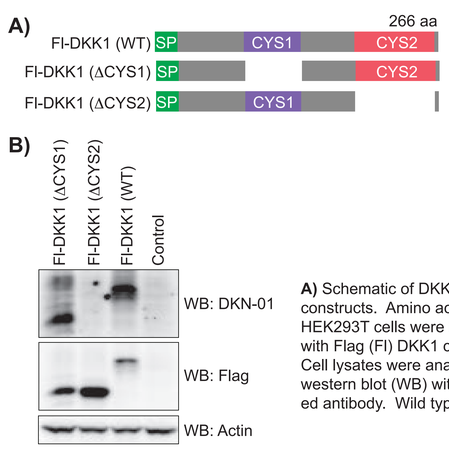
Table 2: DKN-01 is Specific for DKK1

Family Member	K_D
DKK1	3.3 pM
DKK2	Little or no binding up to 1 μM
DKK3	No binding up to 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).

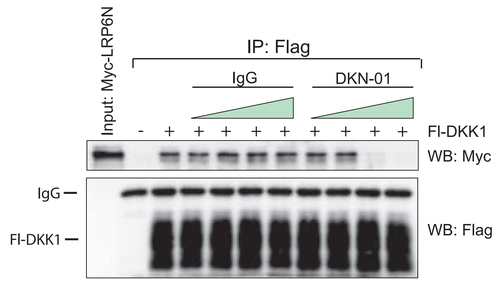
Results

Figure 2: DKN-01 Recognizes the CYS2 Domain of DKK1



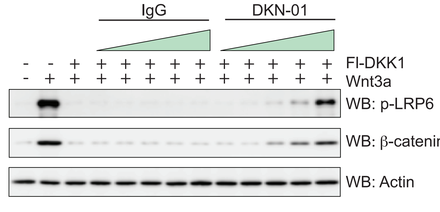
A) Schematic of DKK1 deletion constructs. Amino acid (aa). **B)** HEK293T cells were transfected with Flag (Fl) DKK1 constructs. Cell lysates were analyzed by western blot (WB) with the indicated antibody. Wild type (WT).

Figure 3: DKN-01 Blocks DKK1 Interaction with LRP6



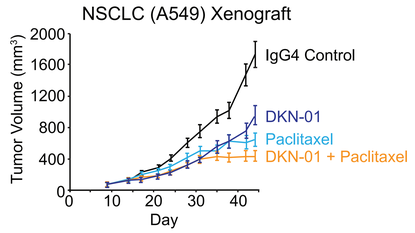
Fl-DKK1 conditioned media was incubated with increasing amounts of DKN-01 or a human IgG control antibody for 1 hour before the addition of Myc-LRP6N conditioned media. Fl-DKK1 was immunoprecipitated (IP) with Fl agarose and coprecipitated LRP6N was detected by western blot (WB). Conditioned media from Fl vector transfected cells was used as a control (second lane from the left).

Figure 4: DKN-01 Neutralizes DKK1 in a Cell Based Assay



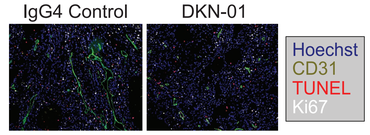
HEK293T cells were stimulated with Wnt3a or combined Wnt3a and Fl-DKK1 conditioned media. Wnt3a and Fl-DKK1 combined conditioned media was pre-incubated with increasing amounts of DKN-01 or a human IgG control antibody. Phosphorylated LRP6 (p-LRP6) and β-catenin levels were measured by western blot (WB) 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).

Figure 5: DKN-01 Has Efficacy in a NSCLC Xenograft



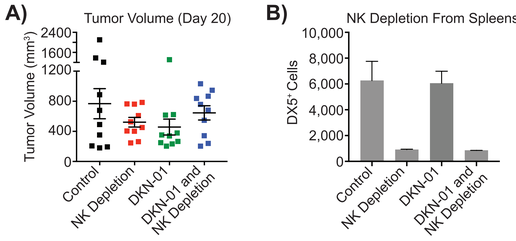
SCID mice were inoculated subcutaneously with A549 cancer cells that were previously validated to secrete DKK1 *in vitro*. Treatment began when tumors reached 75 mm². Groups of 10 mice were dosed with DKN-01 (5 mg/kg, once a week) alone or in combination with paclitaxel (20 mg/kg, twice a week). The control group was dosed with a IgG4 antibody (5 mg/kg, once a week). Non small cell lung cancer (NSCLC).

Figure 6: DKN-01 Decreases Angiogenesis and Proliferation in a NSCLC Xenograft



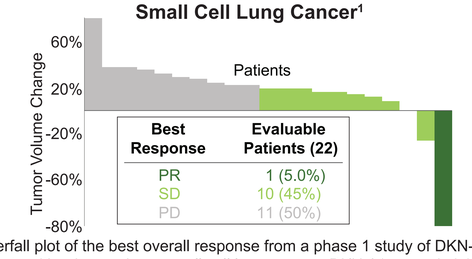
SCID mice were inoculated subcutaneously with A549 cells. Groups of 10 mice were dosed once weekly with DKN-01 (5 mg/kg) or an IgG4 control antibody (5 mg/kg) when the tumors reached 200 mm³. 10 days after the first dose tumors were excised and stained for the indicated markers. Angiogenesis was evaluated by staining for CD31. Cell proliferation was determined by Ki67. Apoptosis was measured by a Terminal UDP Nick-End Labeling (TUNEL) assay. Non small cell lung cancer (NSCLC).

Figure 7: DKN-01 Efficacy May Depend on NK Cells



A) Athymic mice were inoculated subcutaneously with A549 cancer cells. Treatment began when tumors reached ~100 mm². Groups of 10 mice were dosed with DKN-01 (5 mg/kg, once a week) alone or in combination with an anti-asialo GM1 NK cell depleting antibody (dosed every 5 days). Mean and SEM are shown. **B)** Prior to initiating DKN-01 dosing NK cell depletion from spleens (n=2) was verified by Flow 3 Days after the initial anti-asialo GM1 administration. Error bars represent the standard deviation.

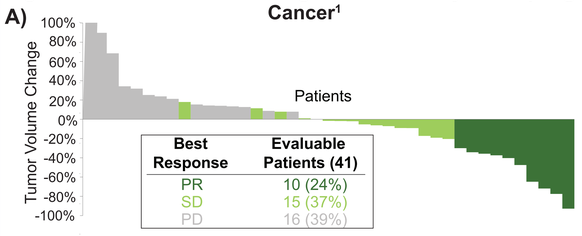
Figure 8: DKN-01 Monotherapy Response in Advanced Non Small Cell Lung Cancer[1]



Waterfall plot of the best overall response from a phase 1 study of DKN-01 in patients with advanced non small cell lung cancer. DKN-01 was administered weekly or biweekly at 75, 150, 300 or 600 mg during a 28-Day cycle for Part A patients. Part B patients received DKN-01 at 300 mg on Day 1 and 15 of a 28-Day cycle. Graph shows the combination of all evaluable Part A and B patients. Partial response (PR). Stable disease (SD). Progressive disease (PD).

[1]ASCO, Abstract #6068, 2014

Figure 9: DKN-01 has Promising Clinical Activity in Esophageal Cancer[1]



A) Waterfall plot of the best overall response from an ongoing phase 1 study of DKN-01 in combination with paclitaxel in patients with advanced esophageal cancer or gastroesophageal junction tumors. Patients received 150 or 300 mg of DKN-01 on Day 1 and 15 and 80 mg/m² of paclitaxel weekly during a 28-Day cycle. Graph shows the combination of all evaluable patients. Partial response (PR). Stable disease (SD). Progressive disease (PD). **B)** Pre and post DKN-01 images from a patient with a PR. Arrow designates tumor location.

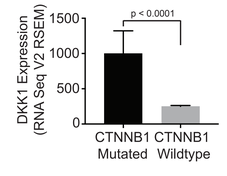
[1]ASCO GI, Abstract #161, 2017

Table 3: Identification of Stabilizing CTNNB1 (β-catenin) Mutations in Esophageal Tumors

Response*	Duration of Study	β-catenin Mutation
PR (-78%)	In Cycle 23	Exon 2-4 deletion
PR (-37%)	4 Cycles	S45F
SD (-7%)	11 Cycles	T41N
PD (8%)	2 Cycles	T41N

Formalin fixed paraffin embedded (FFPE) archived tumor tissue was analyzed for genetic mutations using Next-Generation Sequencing with a Archer VariantPlex Solid Tumor Kit or a FoundationOne test (exon 2-4 deletion patient only). Genetic data was available for 18 evaluable patients, 4 PR (partial response), 8 SD (stable disease) and 6 PD (progressive disease). *11/30/16

Figure 10: DKK1 Expression is Elevated in Tumors with Stabilizing CTNNB1 (β-catenin) Mutations



Publicly available expression and genetic data from diverse tumor types was accessed through cBioPortal. DKK1 expression data for tumors with CTNNB1 mutations or deletions at S33, S37, T41 or S45 (n=106) was compared to tumors without detected mutations or deletions (n=7036). p-value was calculated with an unpaired t test. Error bars represent the SEM.

Conclusions

- DKN-01 is a high affinity selective antibody for DKK1
- DKN-01 has promising clinical activity in a number of malignancies
- CTNNB1 stabilizing mutations may be a biomarker for DKN-01

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