DKN-01 Demonstrates Immune Modulatory Activity and Robust Efficacy in Colorectal Cancer Models

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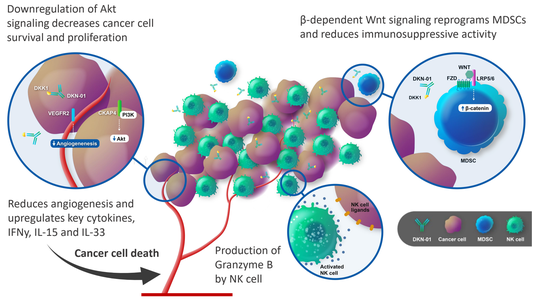
Poster # 1141

BACKGROUND

DKK1

- DKK1 is a secreted protein that plays a modulatory role in Wnt/β-catenin and PI3K/Akt signaling pathways.[1]
- DKK1 promotes tumor growth and contributes to an immunosuppressive tumor microenvironment.[1]
- DKN-01 is a targeted anti-DKK1 mAb that has demonstrated improved clinical outcomes in gastric and gastroesophageal junction adenocarcinoma patients.[2]

DKN-01 Mechanism of Action



Colorectal Cancer (CRC)

- CRC characterized by hyperactivation of the Wnt pathway, often believed to be the initiating and driving event.
- Adenomatous polyposis coli (APC) is the most frequently mutated gene in CRC (~80%) resulting in β-catenin stabilization and subsequent increased expression of DKK1.[3]
- DKK1 has been shown to be associated with resistance to 5-Fluorouracil (5FU), commonly used to treat metastatic CRC.[4]
- Anti-PD-1 therapies have not shown efficacy in microsatellite stable (MSS) CRC.[5] DKN-01 has demonstrated synergy with anti-PD-1 therapies in other indications.[2]

METHODS

Animal Models

- For the xenograft models, athymic nude mice were inoculated subcutaneously (SC) with either parental or 5FU-resistant colon carcinoma cell lines. Once tumors reached 50 mm^3, dosing was initiated with either Isotype control (IgG4; 5 mg/kg, BIW, IP), DKN-01 (5 mg/kg, BIW, IP), 5FU (30 mg/kg, Q2D, IP), or the combination.
- For the CT26 syngeneic model, BALB/c mice were inoculated SC with CT26 murine colon carcinoma cells. Once tumors reached 50 mm^3, dosing initiated with either Isotype control (IgG2a; 10 mg/kg, BIW, IP), a murinized version of DKN-01 (mDKN-01; 10 mg/kg, BIW, IP), anti-PD-1 (10 mg/kg, Q4D, IP), or the combination. Tumors were harvested 14 days post-treatment and both IHC and bulk RNAseq analyses were performed on each tumor.

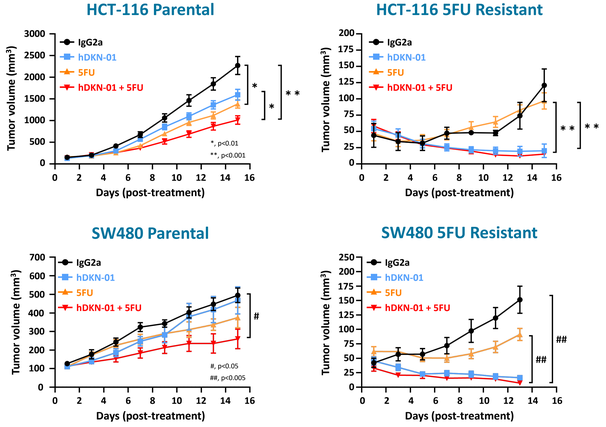
Histology

- Formalin fixed paraffin embedded (FFPE) mouse tumor sections were prepared and stained via IHC. The relative amount of tumor PD-L1, CD3 and CD8 immunoreactivity was estimated in each section by measuring the percentage of the tumor area occupied by brown pixels, using the QuPath "Create Thresholder" function.

RESULTS

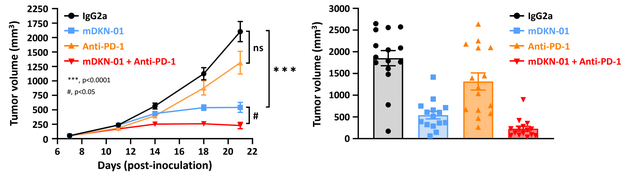
DKN-01 Has Activity in Multiple Xenograft Models Including Those That Are Resistant to 5FU

- DKN-01 has single agent efficacy in CRC xenograft models including HCT-116 and SW480.
- Additive activity was seen with 5FU in both models.
- 5FU resistant cell lines were created from both HCT-116 and SW480 cell lines, reflective of a second line CRC setting.[4]
- In the 5FU-resistant models (right panels), DKN-01 demonstrated significant inhibition of tumor growth.

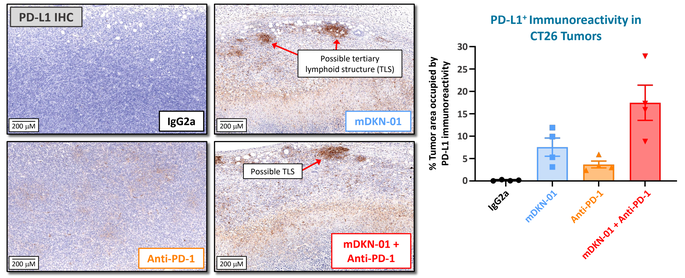


mDKN-01 Has Monotherapy Activity Alone and in Combination with Anti-PD-1 in a Syngeneic CT26 Model

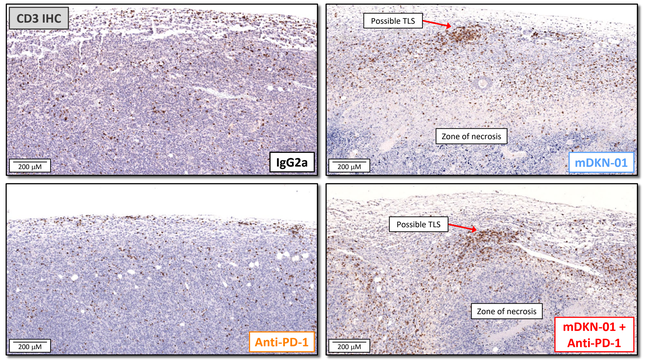
- mDKN-01 also has significant single agent efficacy in the MSS stable CRC syngeneic CT26 model.
- Additive activity was seen with anti-PD-1 with both mDKN-01 and combination treatment leading to tumor regression.



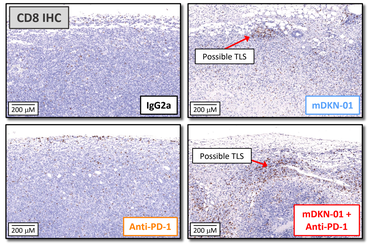
Treatment with mDKN-01 Alone and with Anti-PD-1 Increases PD-L1$^+$ Immunoreactivity in CT26 Tumors



Treatment with mDKN-01 Alone and with Anti-PD-1 Results in Increased T-Cell Infiltration in CT26 Tumors

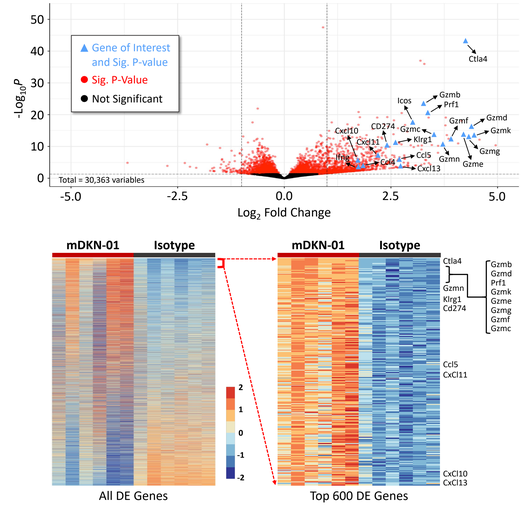


- **Isotype control (IgG2a)** and **anti-PD-1** treated animals show minimal regions of tumor necrosis, widely scattered CD3$^+$ and CD8$^+$ T cells throughout the tumor and minimal presence in adjacent connective tissue.
- **mDKN-01** and **mDKN-01+ anti-PD-1** treated animals have substantial tumor necrosis, robust infiltration of CD3$^+$ and CD8$^+$ T cells along the periphery of the necrotic tumor and adjacent connective tissue.



Bulk RNAseq Analysis on CT26 Tumors

- Preliminary differential gene expression analysis comparing mDKN-01 vs Isotype control treated tumors reveal significant upregulation in cytotoxic and pro-inflammatory gene signatures.



CONCLUSIONS

- DKN-01 shows additive activity with 5FU and can overcome 5FU-resistance in two xenograft models, resulting in tumor regressions.
- 5FU-resistant models are reflective of a second-line CRC population currently being recruited in the DeFianCe study (NCT05480306).
- Treatment with mDKN-01 or in combination with anti-PD-1 results in tumor regression in a CT26 syngeneic model.
- mDKN-01 treatment increases PD-L1 immunoreactivity in a CRC syngeneic CT26 model.
- mDKN-01 used alone or in combination with anti-PD-1 promoted substantial CT26 tumor necrosis which was associated with a robust immune cell infiltrate and possible emergence of tertiary lymphoid structures in the peritumoral stroma.
- The CT26 tumor immune infiltrate observed in mDKN-01 / anti-PD-1 treated animals contains a substantial number of CD3$^+$ and CD8$^+$ cells, implying the presence of an adaptive immune response to tumor antigen.
- Initial bulk RNAseq analysis reveal mDKN-01 monotherapy significantly increases genes responsible for T cell activation, cytotoxic activity and TLS formation.

References: 1. Kagey MH, He X. *Br J Pharmacology*. 2017;174:4637–4650. 2. Klempner SJ, et al. *Mol Cancer Ther*. 2021;11:2240-2249. 3. Fearon ER. *Annu. Rev. Pathol. Mech. Dis.* 2011;6:479–507. 4. Zhao Y, et al. *Carcinogenesis*. 2021;42:814-825. 5. Ganesh K, et al. *Nat Rev Gastroenterol Hepatol*. 2019;16:361–375.

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