Abstract # 1642: EVALUATION OF ENMD-2076 IN COMBINATION WITH ANTI-PD1 IN SYNGENEIC CANCER MODELS

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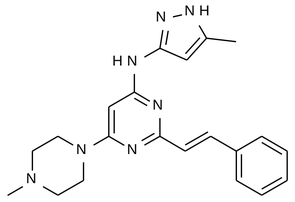
ABSTRACT

ENMD-2076 is a clinical stage compound with potent activity towards Aurora A and angiogenic kinases. ENMD-2076 has shown promising activity in multiple Phase 1 clinical trials, as well as in a Phase 2 trial in advanced ovarian cancer. ENMD-2076 is currently the subject of several ongoing Phase 2 clinical trials including fibrolamellar carcinoma, triple-negative breast cancer (TNBC), advanced/metastatic soft tissue sarcoma (STS), and advanced ovarian clear cell carcinomas (OCCC). ENMD-2076 has been developed to date as a single agent, however ENMD-2076 inhibits a spectrum of targets including Aurora A, FAK, CSF1R, c-Kit, and KDR, that are potentially involved in immune evasion mechanisms. These kinases have been shown in published studies, when inhibited, to enhance or augment the activity of immune checkpoint inhibitors such as anti-PD-1. A study was thereby conducted in syngeneic models to determine the utility of ENMD-2076 combined with immune checkpoint inhibition as a rational strategy for cancer therapy. The study evaluated the efficacy of ENMD-2076 administered daily by oral gavage in the MC38 and CT26 colon cancer models, alone and in combination with an anti-PD-1 antibody. Xenografts were established in the appropriate mouse strain (C57BL/6 and BALBc, respectively) by the subcutaneous inoculation of MC38 or CT26 cells into the right flank of female mice. Treatment was initiated 7 days following inoculation when tumor volumes had reached a mean volume of approximately 85 mm^3. All treatments were well tolerated, with no significant body weight loss seen during either study. While CT26 tumors were relatively refractory to single agent ENMD-2076, tumor regression was observed in several MC38-bearing animals suggesting an immune activating mechanism. In both models a trend was observed for an augmentation of anti-tumor response in combination relative to single agent ENMD-2076 and anti-PD-1 alone. Further studies to evaluate mechanism and an assessment of re-challenge experiments in animals exhibiting complete regression of tumors will be discussed. These studies support the further evaluation of ENMD-2076 in combination with immune checkpoint inhibition as a strategy for cancer therapy.

Figure 1. Structure of ENMD-2076



L-(+)-Tartaric acid

2-(2-Phenylvinyl)-4-[4-methylpiperazin-1-yl)]-6-(5-methyl-2H-pyrazol-3-yl-amino)-pyrimidine L(+) tartrate salt

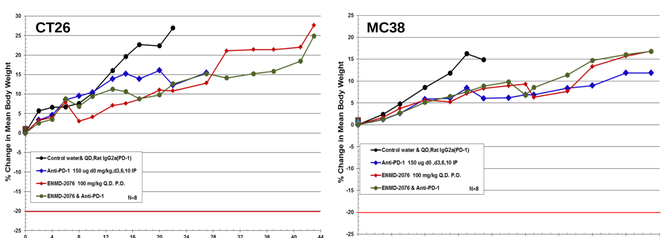
Table 1. ENMD-2076 inhibits multiple targets implicated in immune evasion

ENMD-2076 inhibits multiple kinases that have been shown to be involved in immune-evasion mechanisms, supporting the evaluation of the compound in syngeneic models.

	IC_{50} nM Recombinant Protein
Flt3	3
AurA	14
Src	20
CSF1R	25
KDR/VEGFR2	36
FGFR1	93
cKit	120
FAK/PTK2	55

ENMD-2076 was screened for inhibitory activity against a panel of recombinant kinases in an *in vitro* FRET-based assay. IC_{50}s are shown for the most potent hits relevant to immune evasion mechanisms.

Figure 2. Negligible toxicity observed with combination of ENMD-2076 and anti-PD-1 *in vivo*



Xenografts were established in the appropriate mouse strain (C57BL/6 and BALBc, respectively) by the subcutaneous inoculation of MC38 or CT26 cells into the right flank of female mice. Treatment was initiated 7 days following inoculation when tumor volumes had reached a mean volume of approximately 85 mm^3. Anti-PD-1 antibody, (Clone RMP1-14 (BioXcell)) was given as a 150 µg dose, IP, Day 0, 3, 6, and 10; ENMD-2076, 100 mg/kg oral in water for 21 days. Combinations of anti-PD-1 and ENMD-2076 were dosed as in the individual arms. Rechallenge experiments were performed by inoculating cell lines into the opposite flank of animals exhibiting complete regressions in the original study.

Figure 3. Orally Administered ENMD-2076 induces tumor cell specific immunity alone and in combination with anti-PD-1

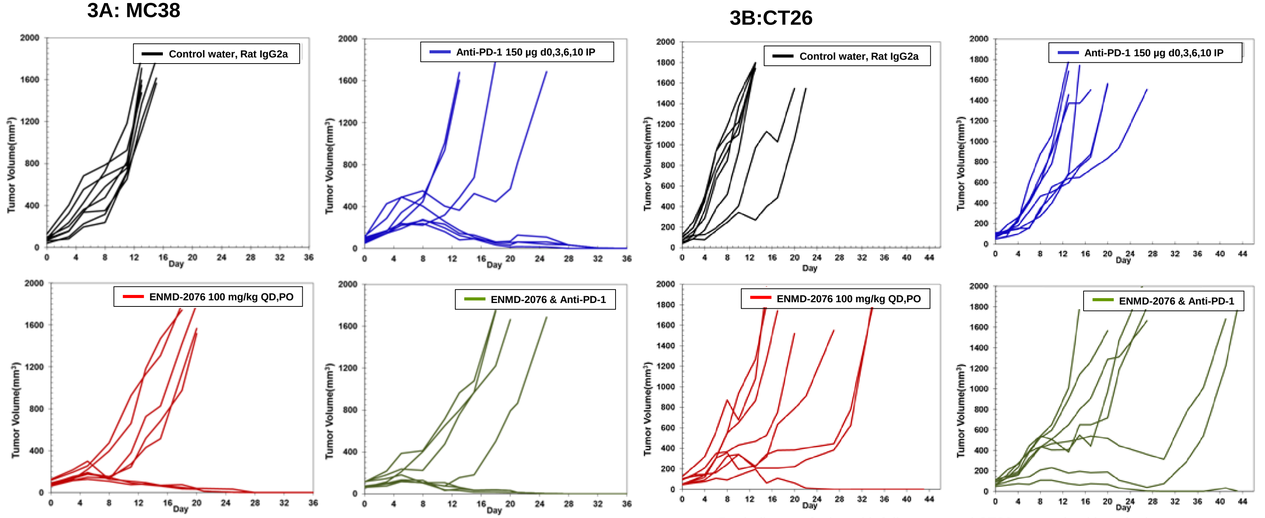
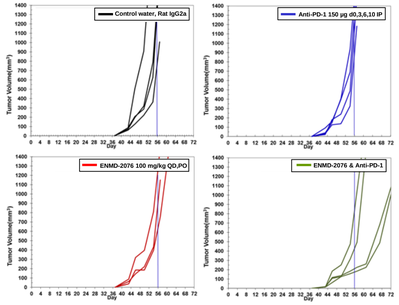


Figure 4. Rechallenge of mice with fully regressed MC38 tumors show delayed growth with ENMD-2076/antiPD-1 combination treatment



Rechallenge of mice with fully regressed CT26 tumors in either single agent ENMD-2076 or combination arms resulted in no tumor growth. Challenge with a different line (4T1) in the same animals resulted in growth of tumors, indicating that the immunity generated was specific to CT26

CONCLUSIONS

- **ENMD-2076 potently inhibits multiple kinases implicated in immune evasion including AurA, KDR, CSF1R, and cKit**
- **ENMD-2076 combines safely with anti-PD-1 therapy**
- **In the MC38 syngeneic tumor model ENMD-2076 induces single-agent complete regressions, and delays tumor growth in rechallenged animals when in combination with anti-PD-1**
- **In the CT26 syngeneic tumor model ENMD-2076 augments anti-tumor response to anti-PD-1 therapy, but also induces CT26-specific immunity as a single agent**
- **Investigations are underway to determine specific mechanisms of immune modulation by ENMD-2076**
- **These studies provide support for the further evaluation of ENMD-2076 in combination with immune checkpoint inhibition as a strategy for cancer therapy.**