Cytotoxic Cannabinoid Analogs for Prevention of Pancreatic Cancer

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Abstract

The development of antineoplastics for the treatment of pancreatic cancer of less toxic and significantly efficient compounds are unmet and are urgently needed. Our group aims to utilize the remarkable properties of rare cannabinoids towards the treatment of cancer and various ailments. The IC_{50} of our modified cannabinoids, CCL-104 and CCL-106 as potential therapeutics for the treatment of pancreatic cancer induced similar or increased cytotoxicity towards pancreatic adeno-carcinoma cancer cell lines compared to the marketed PARP antineoplastic compounds on the current market.

Introduction

Pancreatic cancer

- 3.2% of all new cancer cases
- An estimated 49,830 deaths in 2022 YTD
- Rare cancer: 11.5% 5y survival rate
- Gemcitabine is a 1st line treatment; reported pulmonary toxicity in humans
- Gemcitabine also creates chemoresistance, requiring combinatorial treatments.

Our team has developed a series of synthetic cannabinoids

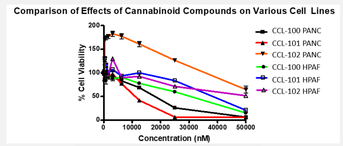
- *In-vitro* and *in-vivo* studies have been completed on various cancer cells
- Traditional Structure v. Activity Relationship scaffolds were also created
- Identified CCL-104 and CCL-106
- These compounds are as toxic to the cancer as traditional treatments (500 nM to 2uM)
- Are safe to human lung, liver, and brain cells *in-vitro*
- They pass the hERG test for cardiac safety and AMES test

Methods

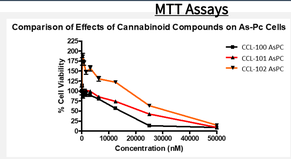
- ***Cell growth inhibition by MTT***: PDAC cells will be seeded in 96-well plates, incubated overnight, then incubated with fresh medium containing drugs at 3 concentrations (1–300 mM). After 72 hours, MTT assay will be performed.
- ***Enzyme inhibition:*** the comparative inhibitory potency of CCL104 analogs against relevant isolated enzymes (tankyrase1, pim1, PARP1, and as appropriate) will be measured using commercial kits. IC50 values will be calculated for selected compounds if significant inhibition is seen.
- **3D Spheroid Assay:** Single-cell suspensions of flow-sorted MiaPaCa-2 CSC spheroids will be plated on ultra-low adherent wells of 6-well plates (Corning) at 1,000 cells per well in sphere formation medium (1:1 DMEM/F12 medium supplemented with B-27 and N-2; Invitrogen). After 7 days, the spheres will be collected by centrifugation (300g, 5 minutes) and counted. The proportion of sphere-generating cells will be calculated by dividing the number of spheres by the number of cells seeded
- **Establish MTD, pharmacokinetics and metabolism of selected compounds** Maximum tolerated dose analysis will be performed to determine the maximum dose of drug that can be given to the mice during the xenograft studies. This is expected to > 60 mg/kg for CCL104. Animals will be dosed at sub MTD dose. Once established, mice will be given the appropriate dose by IV and peripheral blood will be collected at 8 time points (typically 0.083, 0.25, 0.5, 1, 2, 4, 8 and 24 hr.) post-dose (n=3) and analyzed at Akkadian Therapeutics to establish a PK profile for the candidate compounds, using LC-MS/MS methods developed for the purpose.
- **Efficacy of selected CCL-104 analogs in murine xenograft models** Three preferred candidates will be tested in two murine models of xenografts derived from MiaPaCa-2 and patient-derived (PDX) cell lines. Orthogonal and sub-cutaneous xenografts will be induced in mice according to established protocols that are used routinely at Wayne State University[5,6].

Results

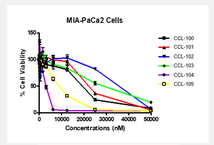
MTT Assays



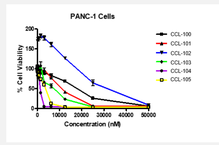
Cell Line	Compound	IC50 (uM)
PANC1	CCL-100	13.116
PANC1	CCL-101	9.038
PANC1	CCL-102	17.253
HPAF-II	CCL-100	7.041
HPAF-II	CCL-101	8.841
HPAF-II	CCL-102	7.023



Cell Line	Compound	IC50 (uM)
AsPC-1	CCL-100	6.523
AsPC-1	CCL-101	16.614
AsPC-1	CCL-102	11.511



Compound	IC50 (uM)
CCL-100	9.7
CCL-101	12.7
CCL-102	27.2
CCL-103	19.7
CCL-104	1.3
CCL-105	6.1



Compound	IC50 (uM)
CCL-100	13.1
CCL-101	9
CCL-102	17.3
CCL-103	5.07
CCL-104	1.06
CCL-105	2.55

Figure 1: MTT Assays performed on PANC-1, AsPC-1, HPAF-II, and MIA-PaCa2 PDAC cell lines, to provide IC50 values of compounds of interest in the treatment of PDAC.

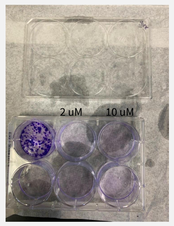


Figure 2: We used 2 uM CCL-104 alone or in the presence of 50 nM Gemcitabine and 1 nM paclitaxel (no colonies were formed

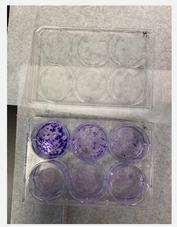
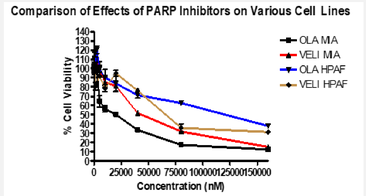
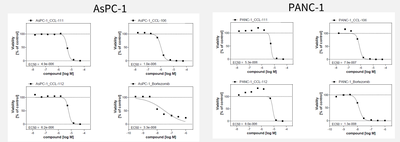


Figure 3: In view of excessive cell killing, we used reduced dose of 1 uM CCL-104 alone or in the presence of 50 nM Gemcitabine and 1 nM paclitaxel



Cell Line	Compound	IC50 (uM)
MIA-PaCa 2	Olaparib	12.805
MIA-PaCa 2	Veliparib	35.04
HPAF-II	Olaparib	58.754
HPAF-II	Veliparib	75.112

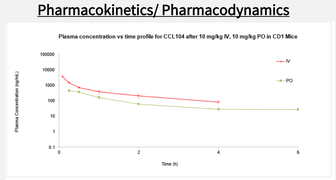
Figure 4: Comparison of currently marketed Poly(ADP-ribose) polymerase inhibitors [PARP], on PDAC cell lines.



Compound	IC 50 (uM) AsPC-1	MiaPaCa-2	PANC-1
CCL-111	4.85	3.24	5.29
CCL-106	1.05	0.53	0.789
CCL-112	6.17	4.77	8.02
Bortezomib	0.03	0.015	0.013

Figure 5: Viability assay was conducted on AsPC-1, MIA-PaCa2, and PANC-1 PDAC cell lines using CCL-106, -111, and -112. Bortezomib was the control

Pharmacokinetics/ Pharmacodynamics



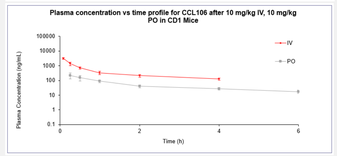
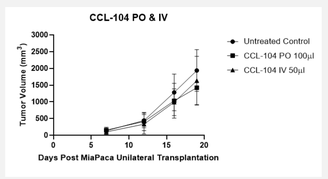


Figure 6: CCL-104 + surfactant oral bioavailability is already 30% without doing any formulation efforts. Oral half-life is 2.6 hours. CCL-106 + surfactant oral bioavailability is 16% without doing any formulation efforts. Oral half-life is 4 hours

Xenograft Models



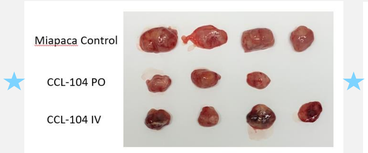
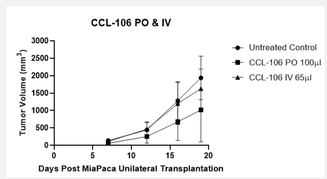


Figure 7: CCL-104 Xenograft study produced tumor shrinkage. Both in PO and IV.



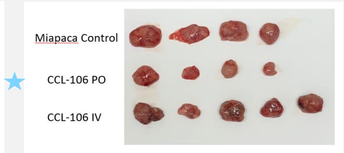
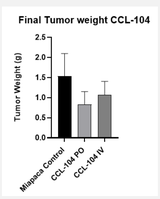
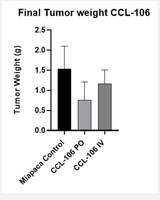


Figure 8: CCL-106 Xenograft study produced significant tumor shrinkage, both in PO and IV and is compound of interest.





Conclusions

- CCL-104 demonstrated cell toxicity to pancreatic cancer cell lines similar to Gemcitibine and Paclitaxel (1-2uM).
- CCL-111 and CCL-112 are not more bioactive than CCL-104, but CCL-106 (IC_{50} = 530 nM - 1000 nM).
- MTD was determined to be 10mg/Kg in mice given CCL-106 and -104.
- CCL-104 and CCL-106 showed a significant reduction in MiaPaCa-2 xenograft models using PO administration.

Future endeavors:

- Repeat MiaPaCa-2 xenograft models
- Complete PANC-1 xenograft model
- Complete a patient derived MiaPaCa-2 xenograft
- Perform GLP toxicity studies
- Push towards IND
- Apply for Orphan Drug Designation

Acknowledgements

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