(250 mL) was added 4-dimethylaminopyridine (88.37 g, 232.69 mmol) and DIPEA (137.7 mL, 775 mmol) and stirred the resulting reaction mass for next 5 minutes followed by the addition of 6-amino-5-fluoropyrimidin-2(1H)-one (1, 20 g, 155 mmol). The resulting reaction mixture was stirred at room temperature for next 16h. After completion of reaction (TLC monitoring), reaction mixture was poured into ice cold water and the precipitate thus obtained was filtered and washed with water (3 x 100 mL), suck dry under vacuum to get the desired compound as off-white solid. Yield 18.0 g (37%).

Synthesis of 9-((5-fluoro-2-oxo-2,3-dihydropyrimidin-4-yl)amino)-9-oxononanoic acid (4): To an ice cold freshly prepared aqueous solution of 5% NaOH (375 mL) was added methyl 9-((5-fluoro-2-oxo-2,3-dihydropyrimidin-4-yl)amino)-9-oxononanoate (**3**, 15 g, 47.92 mmol) portion wise at 0°C. The resulting reaction mixture was stirred at 0°C for next 5-10 min. After completion of the reaction (TLC monitoring), the pH of reaction mass was adjusted up to ~4 by using 6N HCl resulting in the precipitation of off-white solid. Solid material was filtered off and washed with water (3 x 250 mL), suck dried under high vacuum to get the desired product **4** as off-white solid. Yield: 11.0 g (76%).

[00167] Evaluation of the anti-tumor activity of CLX-155 [(2R,3R,4R,5R)-2-(5-fluoro-4-octanamido-2-oxopyrimidin-1(2H)-yl)-5-methyltetrahydrofuran-3,4-diyl diacetate] in relation to Capecitabine in HCT116 xenograft in *Foxn1* athymic nude mice model

Study Code: CLBBI1

The purpose of this study was to evaluate the anti-tumor activity of CLX-155 administered by oral gavage, once daily for 5 days/week (two days off) for three weeks in HCT116 human colon cancer xenograft bearing *Foxn1* athymic nude mice. The anti-tumor activity of CLX-155 was compared with capecitabine.

Foxn1 athymic nude female mice (7-8 weeks) were implanted with human colon cancer cell line, HCT116 (5 million cells/site) subcutaneously in the dorsal right flank. The animals were randomized (N=10) into different treatment groups as vehicle control, CLX-155 (125, 250 and 500 mg/kg/day) or capecitabine (1000 mg/kg/day) based on the tumor volume, when the subcutaneous tumor reached a volume in the range of 70 – 170 mm^3. The doses

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were selected based on the results of a 7-day repeated dose range finding toxicity study in *Foxn1* nude mice.

The animals in the control group were dosed with the formulation used for CLX-155, which consisted of Capryol 90 (32.5% w/v), Polysorbate 80 (2.5% w/v) in Water (q.s) at the dose volume of 8 mL/kg. The Capecitabine formulation was prepared using 0.5% Hydroxypropyl methylcellulose (HPMC E 15) in 40mM citrate buffer, pH 6.0. Treatment to animals was performed for three consecutive weeks (5 days/week dosing followed by 2 days off). The dose volume was 8 and 10 ml/kg body weight for CLX-155 and capecitabine, respectively. The animals were observed for clinical signs (daily), mortality (daily), body weights and tumor volumes (thrice weekly). Tumor growth inhibition (TGI) was calculated based on the tumor volume on a given day as compared to that on day 1.

CLX-155

- CLX-155 showed statistically significant, dose dependent tumor growth inhibition at all doses. The tumor growth inhibition on day 15 were, 57.8, 70.4 and 90.6 % at 125, 250 and 500 mg/kg/day, respectively. Two animals had complete tumor regressions at 500 mg/kg/day.
- There was no mortality in any groups. Mean % body weight reduction were -3.4, -6.4 and -9.9 % at 125, 250 and 500 mg/kg/day respectively.

Capecitabine:

- Capecitabine at 1000 mg/kg/day showed 87.7% tumor growth inhibition.
- Two animals died on day 14 and 22 (one mortality each). The clinical signs of toxicity were hunched back and scaly skin. Mean % body weight reduction was -5.9 %.

CLX-155: Formulations were prepared afresh and administered within one hour after preparation.

- CLX-155 Formulation composition (per ml)

CLX-155 Dose groups	**125 mg/kg**	**250 mg/kg**	**500 mg/kg**	**Vehicle control (0 mg/kg)**
Dose volume	8 ml/kg	8 ml/kg	8 ml/kg	8 ml/kg
CLX-155, Dose concentration, mg	15.625	31.25	62.5	Nil
Capryol 90, mg	325	325	325	325
Polysorbate 80, mg	25	25	25	25

Water q.s. to make, ml	1	1	1	1

- CLX-155 Formulation composition (per 8 ml)

CLX-155 Dose groups	125 mg/kg	250 mg/kg	500 mg/kg	Vehicle control (0 mg/kg)
CLX-SYN-G155-C16, mg	125	250	500	0
Capryol 90, mg	2600	2600	2600	2600
Polysorbate 80, mg	200	200	200	200
Water q.s. to make, ml	8	8	8	8
CLX-155, Dose concentration, mg/mL	15.625	31.25	62.5	Nil

Conclusions:

CLX-155 caused statistically significant, dose dependent tumor growth inhibition at all the dose levels tested. The TGI at 500 mg/kg/day of CLX-155 was comparable to capecitabine at 1000 mg/kg/day. Additionally, complete regression of tumors was seen in 2 /10 animals at 500 mg/kg/day of CLX-155.

The TGI in Capecitabine group at 1000 mg/kg/day was comparable to that observed at 500 mg/kg/day of CLX-155. Capecitabine was relatively more toxic than CLX-155, based on mortality and clinical signs of toxicity.

Tumor Growth Inhibition (TGI)

Group #	Treatment	Percent Tumor Growth Inhibition (% TGI) on days					
		3	5	8	10	12	15
2	CLX-155, 125 mg/kg/day	49.4	59.5	59.8	56.3	58.5	57.8
3	CLX-155, 250 mg/kg/day	83.6	61.4	67.7	66.5	66.7	70.4
4	CLX-155, 500 mg/kg/day	51.1	67.1	75.3	75.4	79.5	90.6
5	Capecitabine, 1000 mg/kg/day	27.3	46.2	68.0	75.4	80.9	87.7

The average initial tumor volume of HCT116 tumor xenografts on day 1 in all experimental

groups was approximately 130 ± 32 mm3 (± standard deviation). The average tumor volume of vehicle control on day 15 was 1259 ± 558 mm3 with nearly 10-fold increase to initial tumor volume.

- All treatments have resulted in a statistically significant inhibition of tumor growth as compared to the vehicle control.
- A dose-dependent inhibition of tumor growth was seen with CLX-155 treatment.
- In CLX-155, 500 mg/kg treatment group, 2/10 animals were complete responders (CR).
- In Capecitabine treatment, there were no CR or PR observed. However, there was 2/10 mortality in capecitabine dosed group.

EQUIVALENTS

[00168] The present disclosure provides among other things compositions and methods for treating cancer and their complications. While specific embodiments of the subject disclosure have been discussed, the above specification is illustrative and not restrictive. Many variations of the systems and methods herein will become apparent to those skilled in the art upon review of this specification. The full scope of the claimed systems and methods should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

INCORPORATION BY REFERENCE

[00169] All publications and patents mentioned herein, including those items listed above, are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.