

## Supporting Information

### **6-Substituted hexamethylene amiloride (HMA) derivatives as potent and selective inhibitors of the human urokinase plasminogen activator for use in cancer**

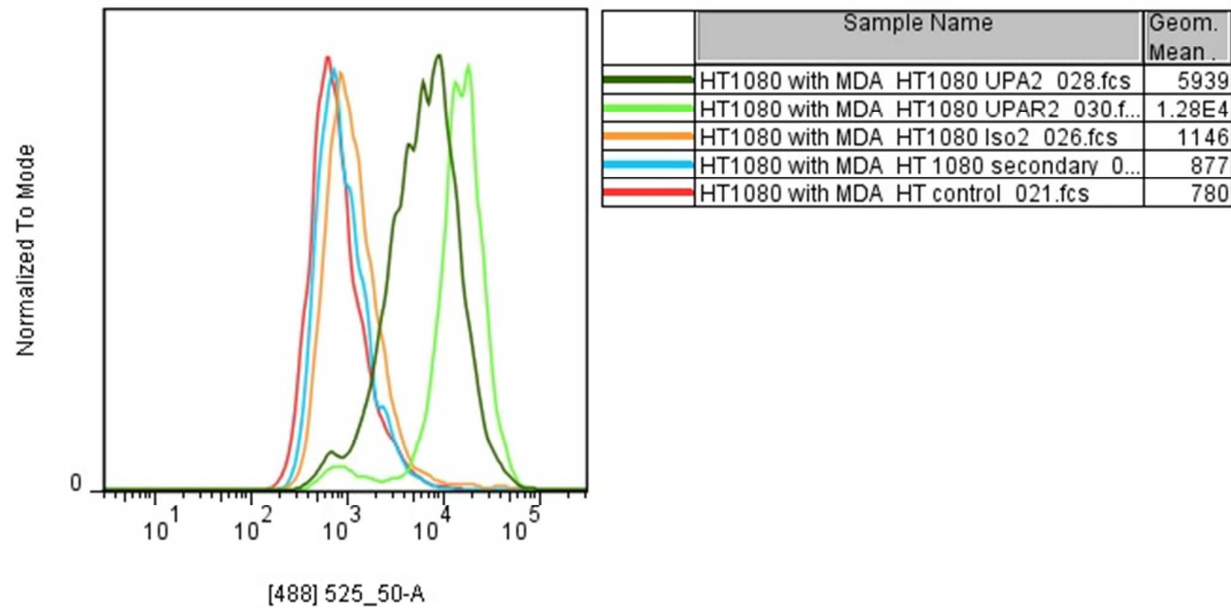
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**Table S1.** X-ray data and model statistics.

Data set/PDB ID	5/5ZA7	14/5ZA8	18/5ZA9	23/5ZAF	22/5ZAE	25/5ZAG	19/5ZC5	26/5ZAH	27/5ZAJ
Space group	R 3	R 3	R 3	R 3	R 3	R 3	R 3	R 3	R 3
Cell dimensions (a,b,c)	120.462	120.768	120.51	120.851	120.33	120.66	120.698	120.434	120.35
	120.462	120.768	120.51	120.851	120.33	120.66	120.698	120.434	120.35
	42.355	42.717	42.403	42.864	42.59	42.336	42.769	42.227	42.28
Resolution range (Å)	34.8 - 1.7	60.4 - 1.9	60.26 - 1.62	60.43 - 1.65	23.92 -	60.33 - 1.95	60.35 - 1.9	60.22 -	28.82 -
	(1.76 - 1.70)	(1.97 - 1.90)	(1.68 - 1.62)	(1.71 - 1.65)	1.73 (1.79 -	(2.02 - 1.95)	(1.97 -	2.98 (3.09	1.65 (1.71 -
					1.73)		1.90)	- 2.98)	1.65)
Total reflections									
Multiplicity	3.8 (3.8)	5.1 (5.1)	3.6 (3.6)	5.1 (5.0)	3.6 (3.8)	2.6 (2.5)	5.0 (4.7)	4.9 (5.1)	3.7 (3.7)
Unique reflections	24667 (2487)	18286 (1828)	28904	28054 (2816)	23162	16793 (1679)	18272	4643 (471)	27316
			(2839)		(2419)		(1839)		(2722)
Completeness (%)									
Overall (final shell)	97.41	99.83	98.82	99.93	96.46	99.89 (99.41)	99.80	99.98	99.45
	(98.07)	(98.86)	(97.83)	(100.00)	(99.88)		(99.78)	(99.79)	(99.82)
R <sub>merge</sub> (final shell)	0.076	0.082	0.066	0.055	0.068	0.091	0.121	0.142	0.091
	(0.691)	(0.553)	(0.973)	(0.522)	(0.686)	(0.591)	(0.699)	(0.628)	(0.077)
R factor	0.1977	0.1993	0.2166	0.2046	0.2394	0.1958	0.2031	0.1678	0.2115
	(0.2801)	(0.2486)	(0.3164)	(0.2691)	(0.2738)	(0.2591)	(0.2376)	(0.2606)	(0.2886)
R <sub>free</sub>	0.2268	0.2327	0.2355	0.2358	0.2760	0.2252	0.2347	0.2680	0.2387
	(0.3166)	(0.2306)	(0.3314)	(0.3037)	(0.3553)	(0.2541)	(0.2722)	(0.3668)	(0.3042)
RMS deviations from ideality									
Bonds (Å)	0.101	0.013	0.022	0.010	0.017	0.018	0.027	0.015	0.017
Angles (°)	3.17	1.51	1.77	1.43	1.87	1.45	1.58	1.62	1.72
Average B factors (Å <sup>2</sup> )									
Protein	29.50	33.70	28.10	27.40	26.50	37.70	37.90	66.40	25.10
Ligands	29.60	37.20	31.10	28.20	28.70	38.60	36.20	83.60	30.10
Solvent	36.20	38.30	39.90	37.20	35.00	39.40	38.40	43.60	37.10



**Figure S1.** uPA and uPAR expression by HT-1080RedFluc human fibrosarcoma cells as determined using flow cytometry. Cells were cultured to ~80% confluence and harvested using 5 mM EDTA. Cells were incubated with 10 µg/mL of mouse anti-human uPA primary antibody (American Diagnostica, #394, light green), mouse anti-human uPAR primary antibody (Dako, M7294, dark green) or mouse IgG isotype antibody (Millipore, #MABC002, orange) with detection using Alexa488-conjugated donkey anti-mouse IgG Fab'2 secondary antibody (Abcam, #ab15010). Cells incubated with the secondary antibody (blue) or no antibody (red) were included as controls. Representative experiment shown.

AE 17/08 Monitoring score sheet (Anti-cancer effectiveness of newly patented amiloride analogues in mouse models of invasive cancer: proof-of-concept study) V1.18082017

	Score 0	Score 1	Score 2	Score 3	Score 4
Wt loss	Wt loss <5%	>5% <10%	>10 <15%	>15% (#acute)	WL >20% *
Posture	Normal	Hunching at rest only	Hunching at rest and movement	Severe /marked hunching (usually accompanied by ruffled coat/grimace etc)	
Activity	Normal	Mild to moderately decreased	Significantly decreased	Stationary unless stimulated or immobile	
Respiration	Normal	Mildly increased rate +/- shallow	Increased abdominal or thoracic effort intermittently or sustained tachypnoea.	Noticeable increased respiratory effort /laboured breathing *	
Hydration	Normal	Skin less elastic (slightly tacky)	Skin tenting mild (returns in <2 seconds)	Skin tenting moderate <3seconds	Skin tenting and eyes sunken. *
Body condition score	BCS 3or 4	BCS 5(obese)		BCS 2 (underconditioned)	BCS 3 (emaciated) *

\*endpoint reached if has any of these  
 #endpoint reached is weight loss is >15% of the maximum over the preceding week.  
 Animals score 3 in any category should be as a minimum assessed by the AWO or Technical officers and scored again later that day .  
**Animals scoring > 9 cumulative MUST be euthanased.**

Acute weight loss is defined as weight loss in a 7 day period. To calculate the percentage acute weight loss the maximum weight over the preceding 6 days is multiplied by 0.85 and if that days weight is less than the calculated weight then the 15% limit has been exceeded.

Example

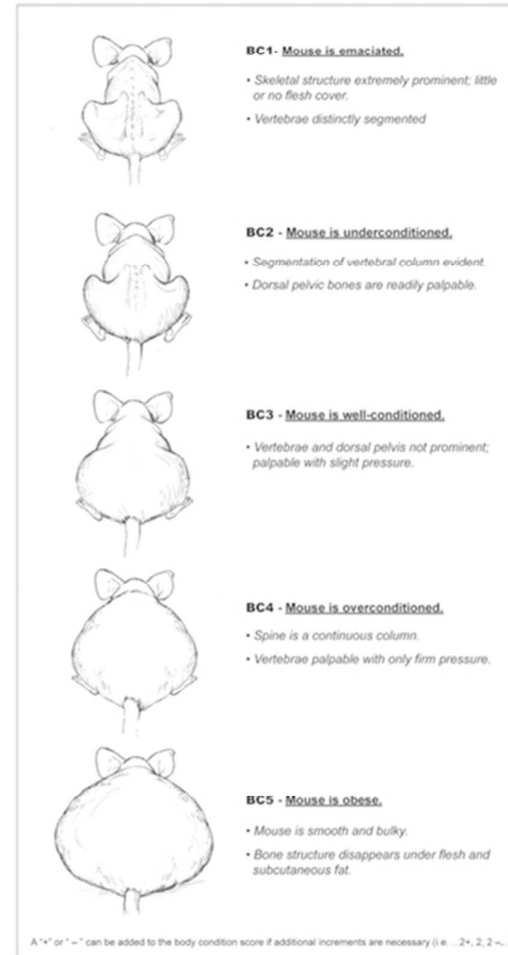
Day 1 = 22.34, Day 2 = 22.37, Day 3 = 23.10, Day 4= 22.4, Day 5= 21.0, Day 6= 20.5  
 Day 7 (19.5) Maximum previous 6 days = 23.10 x0.85 = 19.63g therefore Day 7 weight is greater than 15% loss from Day 3 so acute weight loss endpoint is reached.

Chronic weight loss would be calculated as maximum weight over the protocol x0.80 so in this case if we assume the maximum weight is day 3 for the protocol then the chronic weight loss limit is 23.1 x0.8 = 18.48grams.

Given that mice with tumours can develop ascities and pleural effusions they can gain weight whilst still losing condition so weight loss cannot be the sole criteria for a humane endpoint but needs to be accompanied by other criteria such as activity, posture, respiration, hydration and Body condition score.

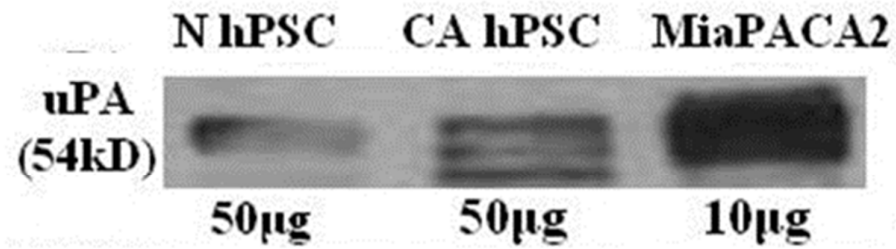
<http://www.iacuc.ucsf.edu/Policies/awSPBodyCondScore.asp>  
<http://ko.cwru.edu/references/Foltz.pdf>

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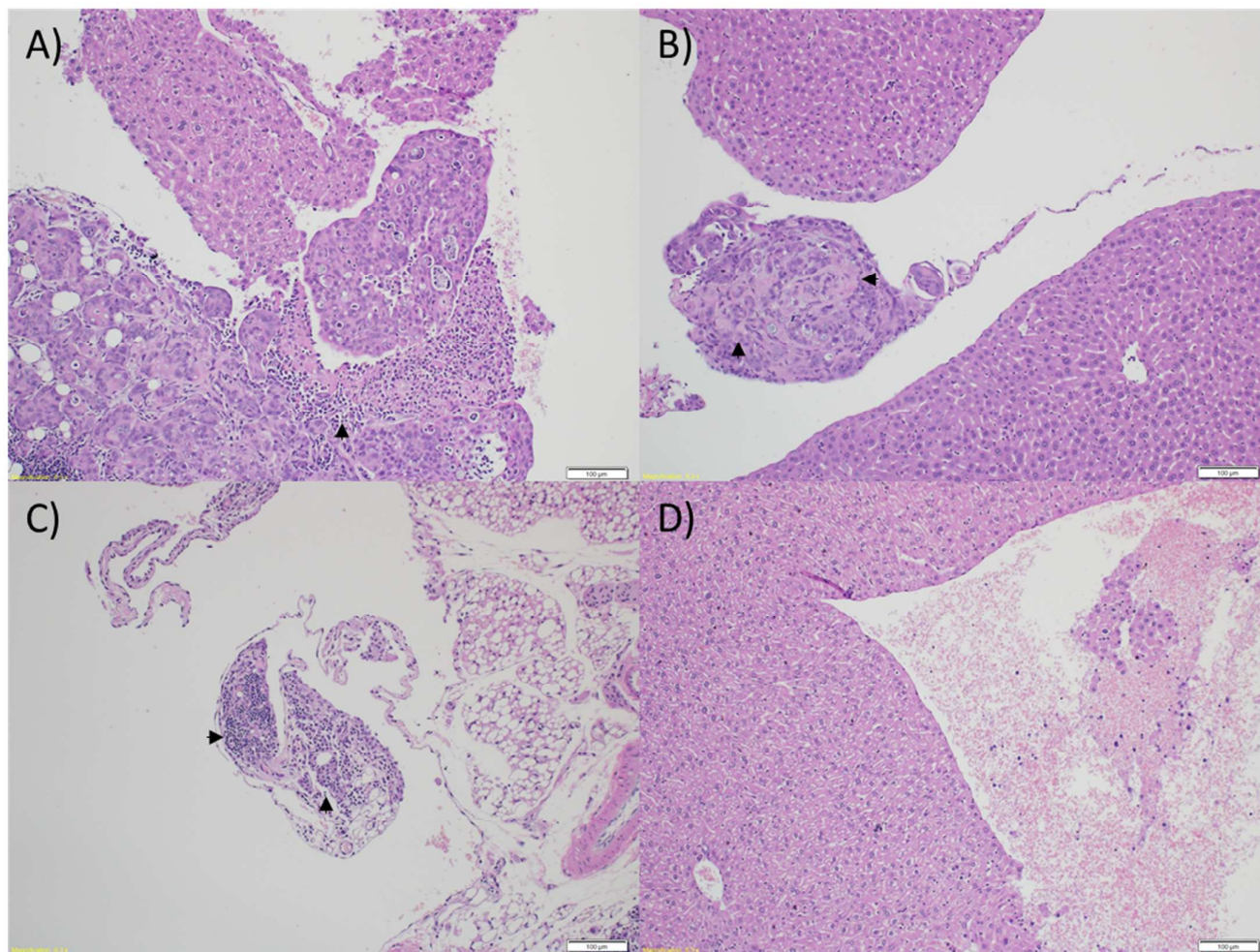


Body condition scoring is a quick and easy methodology that is useful in assessing animal health. It is particularly helpful when body weight might not reflect body condition (e.g. presence of tumors, ascites, organomegaly, pregnancy). Simply run your finger over the sacral area and score the animal according to the chart.

**Figure S2.** Animal condition score sheet for late-stage experimental metastasis model.



**Figure S3.** Western blot of human primary pancreatic stellate cell extracts showing expression of uPA. NhPSC = Normal human pancreatic stellate cells, CAhPSC = Cancer-associated human pancreatic stellate cells. MiaPACA2 = Pancreatic carcinoma cell line.



**Figure S4.** Representative haematoxylin and eosin stained liver sections from pancreatic xenograft mouse model. A) Vehicle. B) Gemcitabine. C) **18**. D) **26**. Arrowheads denote epithelial metastases.