Supporting Information

Tumour regression and improved gastrointestinal tolerability from controlled release of SN-38 from novel polyoxazoline-modified dendrimers

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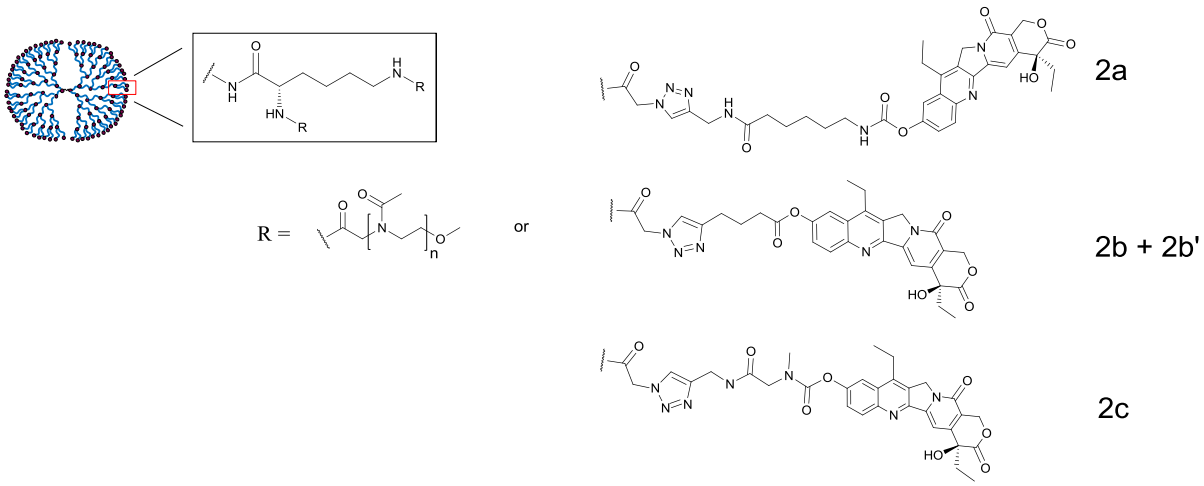

Scheme S1. Linker structures after conjugation to the dendrimer by click chemistry (2a-c) or by amide coupling of the pre-clicked linker-SN38 molecule.

Table S1. HPLC-UV run parameters

HPLC system	HP/Agilent 1100		
Column	Atlantis dC18 5 µm 4.6 x 150 mm		
Solvent A	100% Water + 0.1% Trifluoroacetic acid		
Solvent B	100% Acetonitrile + 0.1% Trifluoroacetic acid		
Solvent C	0.1% Trifluoroacetic acid		
Gradient	Time (min)	**% A**	**%B**
	0	80	20
	0.4	80	20
	2.0	50	50
	4.0	50	50
	5.0	20	80
	6.0	20	80
Flow	1 ml/min		

Table S2. Mass Specrometer and UPLC system parameters

Mass Spec	Waters Xevo TQS		
UPLC system	Water s Acquity i-Class		
Column	Waters Acquity C18 BEH 50 x 2.1, 1.7u		
Solvent A	95% Water, 5% MeOH + 0.1% Formic acid		
Solvent B	95% MeOH, 5% Water + 0.1 % Formic acid		
Gradient	Time (min)	**% A**	**%B**
	0	95	5
	0.3	95	5
	2.2	5	95
	2.6	5	95
	2.61	95	5
	2.8	95	5
Flow	0.6 ml/min		
Run time	2.8 min, use a divert valve for initial 0.5 minutes		

Table S3. Optimization parameters for mass spectrometry analysis

Compound	Ionization mode	Polarity	Parent ion	Daughter ion	Cone voltage (v)	Collison Energy	Retention Time (min)
SN-38	ESI	Positive	393.14	249.15	40	52	1.74
Irinotecan	ESI	Positive	588.08	124.20	10	34	1.41
AZ10024306 (IS)	ESI	Positive	405.11	174.23	80	22	1.59

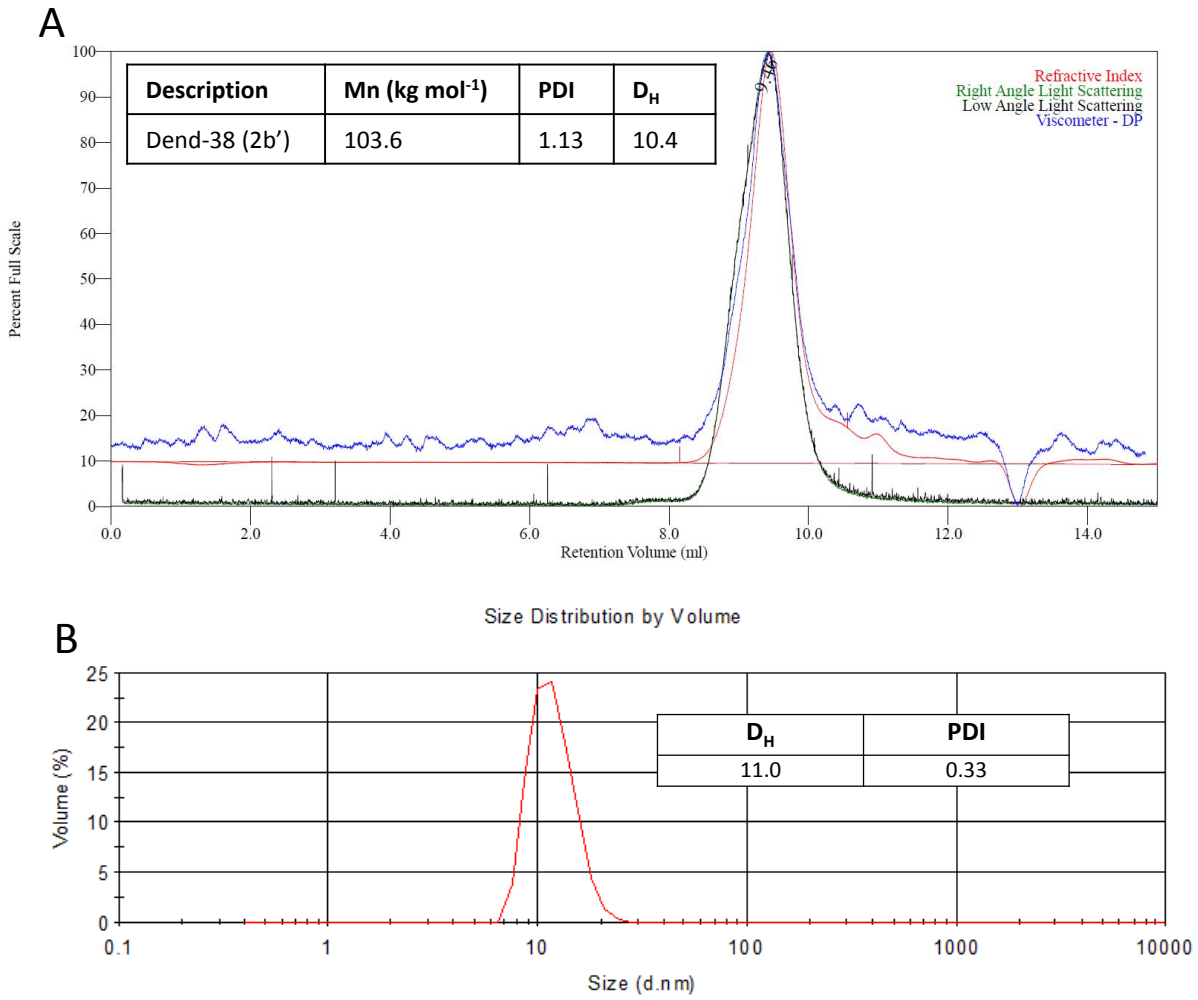

Figure S1. A = Gel Permeation Chromatography-Triple Detection Array chromatogram and molecular weight and calculated data for DEND-38 (2b'). B = Dynamic Light Scattering spectrum showing volume weighted average hydrodynamic diameter in PBS (5 mg/ml).

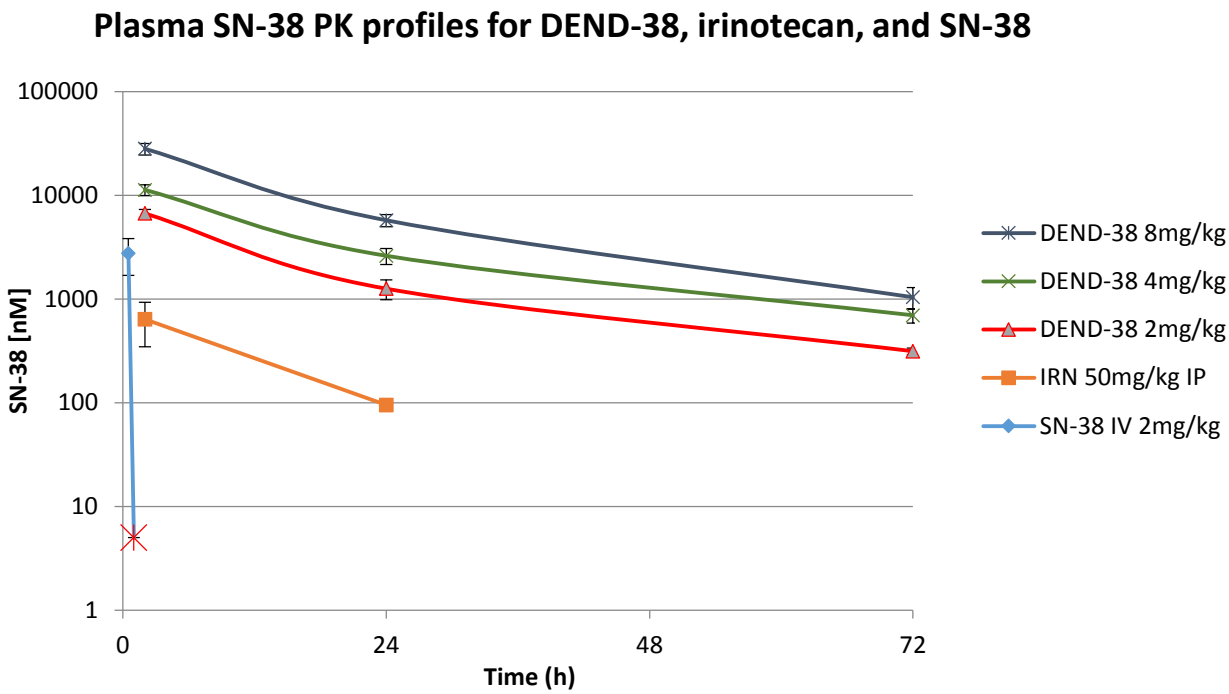

Figure S2. Plasma SN-38 PK data showing total levels (free SN-38 plus conjugated SN-38) for the dose escalation study. SN-38 was dosed as the sodium carboxylate form. (Red cross for SN-38 at 15 min represents that the SN-38 was at the limit of detection of 5 nM in 2/4 plasma samples).

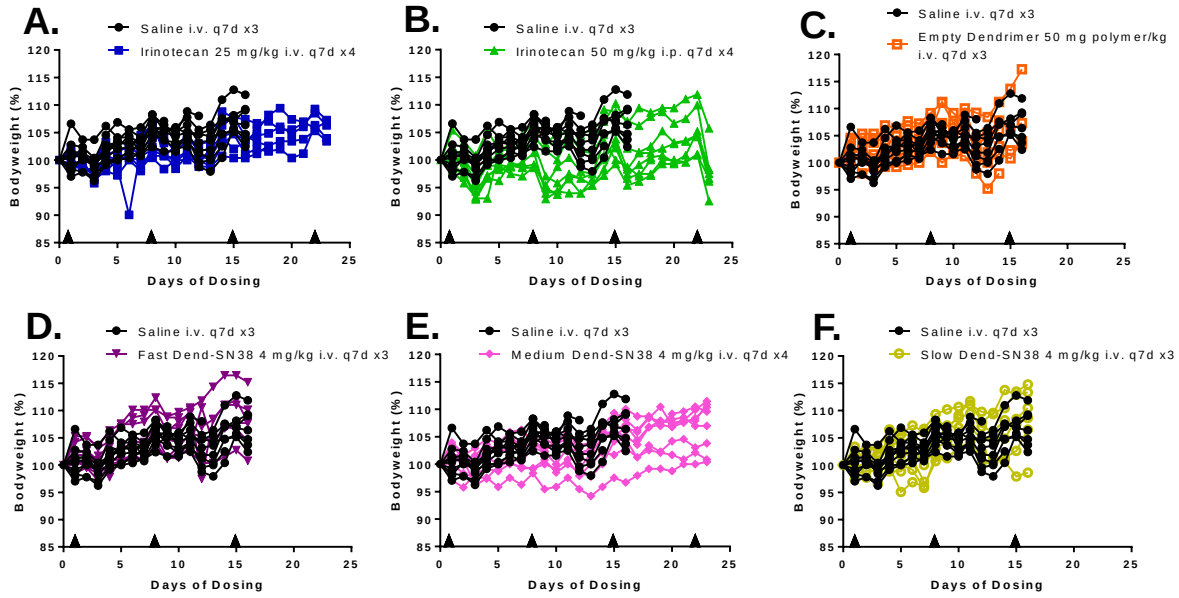

Figure S3. Individual mouse bodyweights throughout study comparing efficacy and gastrointestinal toxicity of Dend-SN38 conjugates (2a-c) versus irinotecan both i.p. and i.v. at the maximum tolerated dose. (A) Saline and irinotecan i.v. (B) Saline and irinotecan i.p. (C) Saline and empty dendrimer. (D) Saline and fast release Dend-SN38. (E) Saline and medium release Dend-SN38. (F) Saline and slow release Dend-SN38.

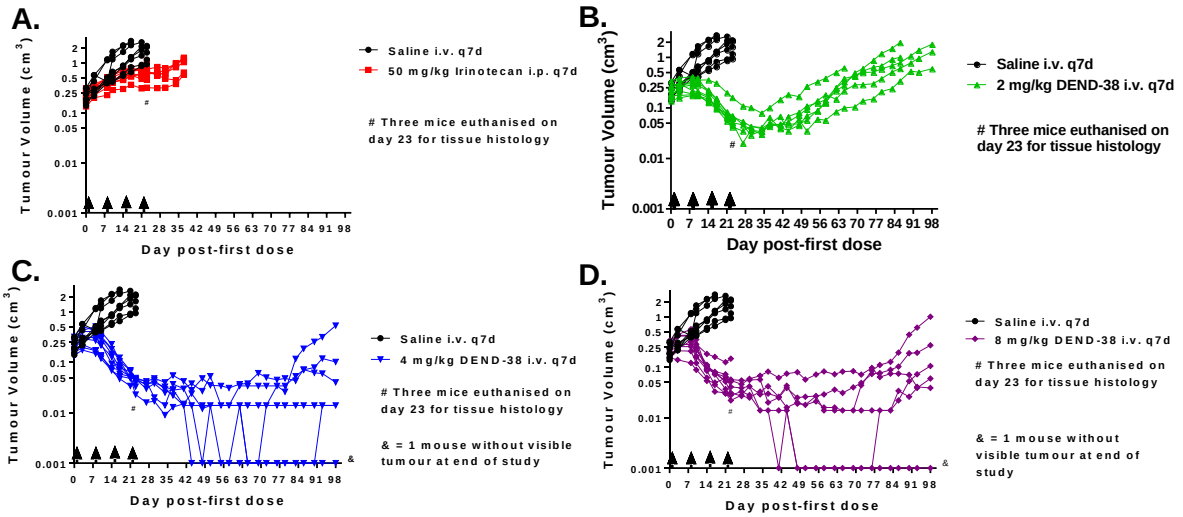

Figure S4. Individual mouse tumour growth curves for study evaluating the efficacy of DEND-38 administered at 2, 4, or 8 mg/kg i.v. q7d. (A) Saline and 50 mg/kg irinotecan i.p. (B) Saline and 2 mg/kg DEND-38. (C) Saline and 4 mg/kg DEND-38. (D) Saline and 8 mg/kg DEND-38.

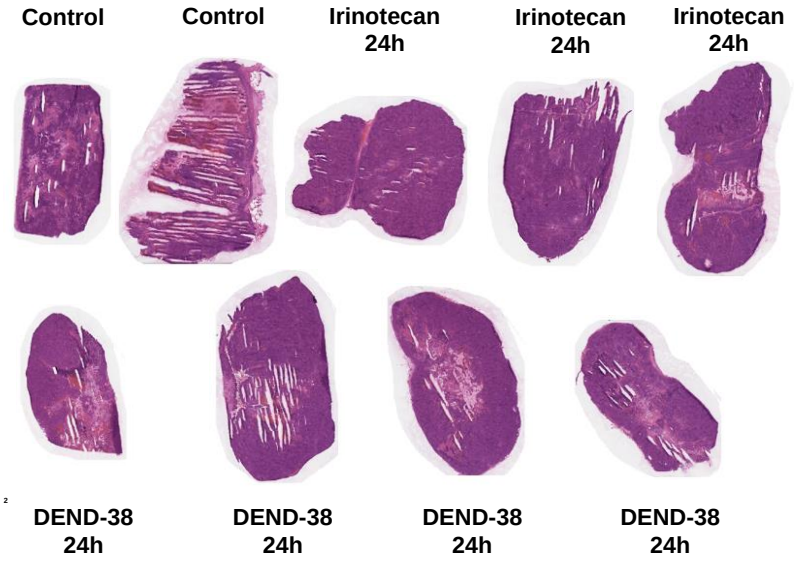

Figure S5. Tumour sections used for DESI-MSI 24 h post-dose of irinotecan i.p. 50 mg/kg or DEND-38 4 mg/kg.

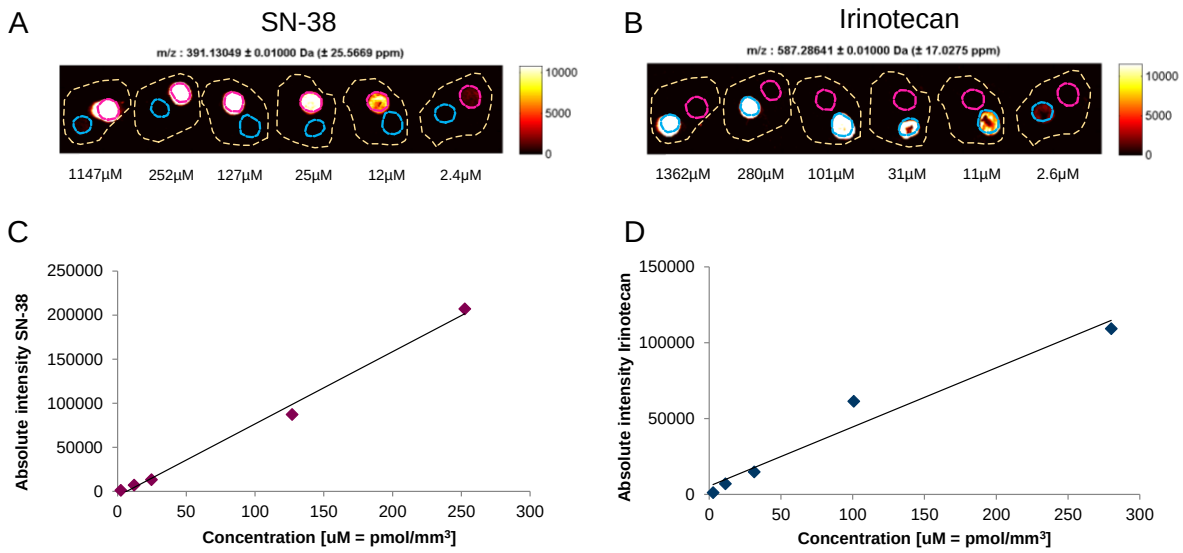

Figure S6. DESI-MS experimentation to explore the sensitivity for irinotecan and SN-38 spiked tumour tissue. A/B = Intensity imaging of tumour sections after spiking with either SN-38 or Irinotecan. C/D = plots of the intensity versus the deposited concentration.