Supplemental Materials

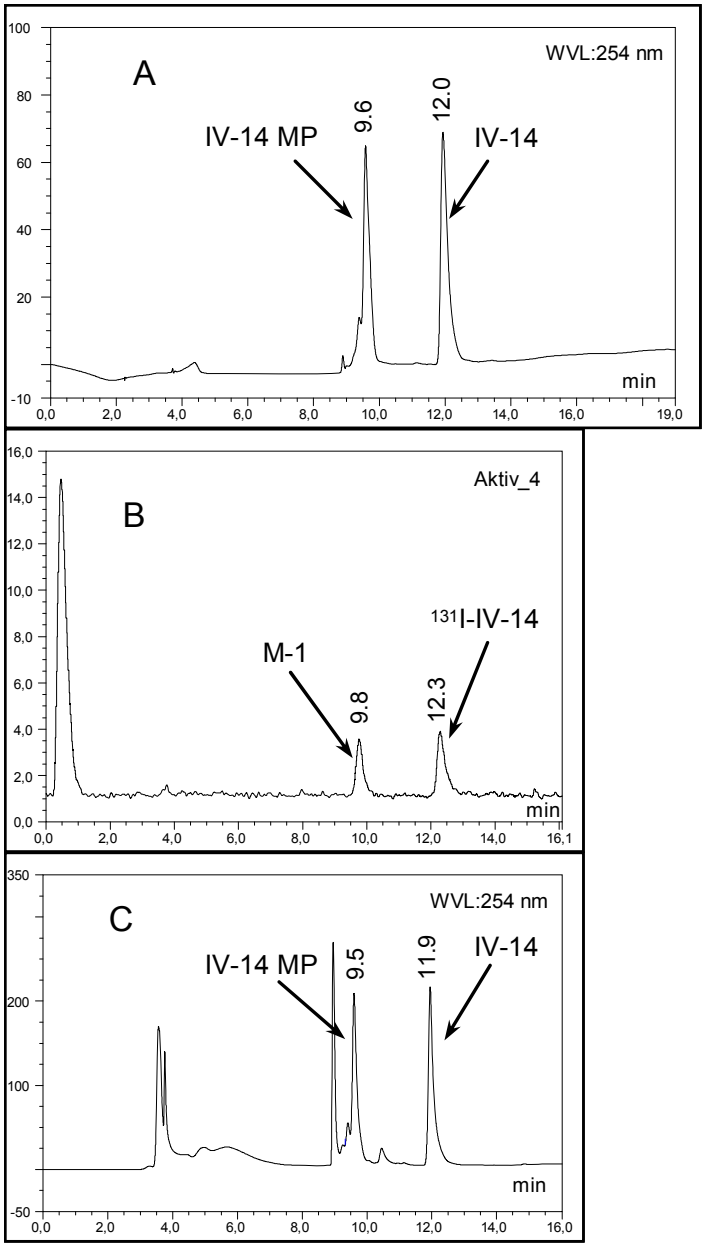
3'-(2-(*E*)-tributylstannylvinyl)uridine (TBSVU). ^{1}H-NMR($CDCl_3$, 500 MHz): δ = 0.61–1.01 (m, 15 H), 1.19–1.33 (m, 6 H), 1.41–1.55 (m, 6 H), 2.81–2.88 (br, 1 H), 3.36–3.40 (br, 1 H), 3.63–3.70 (m, 1 H), 3.75–3.84 (m, 1 H), 4.03–4.08 (br, 1 H), 4.44–4.54 (m, 2 H), 5.72 (d, *J* = 8.0 Hz, 1 H), 5.81 (d, *J* = 7.2 Hz, 1 H), 6.19 (d, *J* = 19.3 Hz, 1 H), 6.61 (d, *J* = 19.3 Hz, 1 H), 7.81 (d, *J* = 8.0 Hz, 1 H), 9.74–9.85 (br, 1 H); ^{13}C-NMR($CDCl_3$, 125.77 MHz): δ = 9.6, 10.7, 13.7, 27.2, 29.1, 62.1, 81.6, 88.3, 91.5, 102.3, 132.1, 142.2, 144.5, 151.6, 163.9. MS (ESI): positive mode m/z = 1143.4 ($[2M + Na]^+$), 561.2 ($[M + H]^+$); ESI HRMS: calculated for $C_{23}H_{40}N_2O_6SnNa^+$: 583.18009; found: 583.17990; calculated for $C_{23}H_{41}N_2O_6Sn^+$: 561.19815; found: 561.19786.

3'-(2-(*E*)-iodovinyl)uridine (IV-14). ^{1}H-NMR[$(CD_3)_2CO$, 500 MHz]: δ = 3.57–3.66 (m, 1 H), 3.75–3.82 (m, 1 H), 3.92–4.01 (br, 1 H), 4.30–4.38 (br, 1 H), 4.46 (d, *J* = 6.8 Hz, 1 H), 4.64 (t, *J* = 3.6 Hz, 1 H), 4.90–5.05 (br, 1 H), 5.63 (d, *J* = 8.1 Hz, 1 H), 6.06 (d, *J* = 7.7 Hz, 1 H), 6.70 (d, *J* = 14.3 Hz, 1 H), 6.94 (d, *J* = 14.3 Hz, 1 H), 8.12 (d, *J* = 8.1 Hz, 1 H), 9.88–10.15 (br, 1 H); ^{13}C-NMR[$(CD_3)_2CO$ 125.77 MHz): δ = 62.3, 77.4, 79.6, 83.7, 88.1, 88.4, 103.0, 142.1, 146.4, 152.0, 163.5. MS (ESI): positive mode m/z = 419.0 ($[M + Na]^+$), 397.0 ($[M + H]^+$); ESI HRMS: calculated for $C_{11}H_{14}IN_2O_6^+$: 396.98911; found: 396.98920.

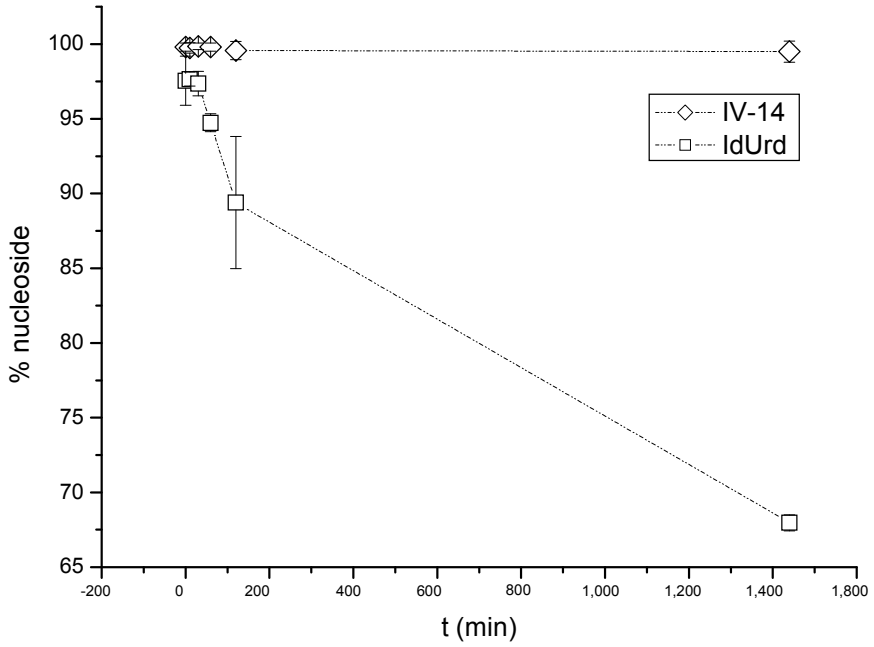
Preparation of liver and small intestine mucosa homogenates. The small intestine and the liver of C.B-17 SCID mice were resected immediately after decapitation. After rinsing with ice-cold 0.9% NaCl the small intestine was divided longitudinally and the mucosa was scraped off with a microscopy slide. Mucosa and liver were homogenized with 3 volumes of 20 mM potassium phosphate buffer (pH 8.0) containing 1 mM EDTA, 2 mM 2-mercaptoetahol and 1 mM PMSF by Micro-Tissue homogenizer (Wheaton, Millville, USA). Cellular debris was removed by ultracentrifugation at 100000 × g and 4°C for 1 h. The clear supernatant was transferred into filter devices with cut-off 30 kDa (Microcon YM-30, Millipore, Bedford, USA) and subsequently centrifuged for 30 min at 12000 rpm at 4°C. Determination of protein concentration was carried out by using Bradford Reagent (Sigma) (*12*). Directly before use the homogenates were transferred into filter devices with cut-off 3 kDa (Microcon YM-3, Millipore, Bedford, USA) and washed three times from the low-molecular impurities by dilution with 9 volumes of 20 mM potassium phosphate buffer (pH 8.0) containing 1 mM

EDTA, 2 mM 2-Mercaptoetahol and concentration to initial volume by centrifugation as described above.

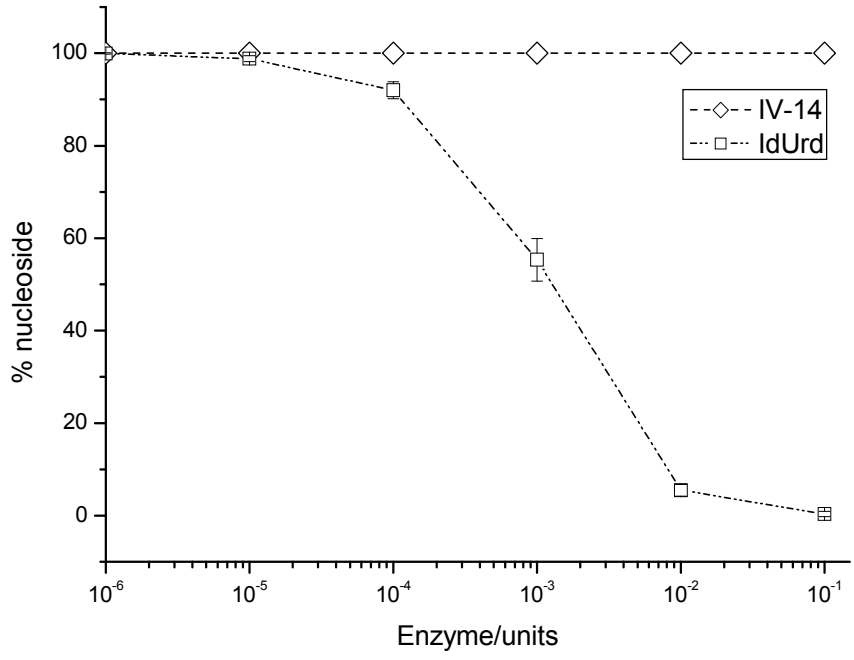
Synthesis of 3'-(2-(*E*)-iodovinyl)uridine-5'-monophosphate (IV-14 MP). $POCl_3$ (10 μL, 16.8 mg, 0.109 mmol) was added to an ice-cold vigorously stirred solution of IV-14 (30 mg, 0.076 mmol) and Proton Sponge (25 mg, 0.114 mmol) in trimethylphosphate (0.4 mL; dried over 10 Å molecular sieves) and the reaction mixture was stirred for 2 h at the same temperature. Thereafter, 0.2 M $(Et_3NH)HCO_3$ (2.5 mL) was added to the reaction mixture and stirring continued for several minutes. The mixture was allowed to reach an ambient temperature and then stirred for a further 45 min. Water (10 mL) was added and the aqueous solution was extracted with Et_2O (8 × 10 mL), concentrated to ca. 5 mL under reduced pressure and lyophilized to give a colorless hygroscopic solid which was dissolved in water (5 mL) and acidified with Amberlite IRA120 to pH=2 and the resin was filtered off. The solution contained IV-14 and IV-14 MP in ratio 94:6 together with Proton Sponge and minor impurities. IV-14 MP was separated by semi-preparative HPLC: eluent A: water (0.1% TFA), eluent B: acetonitrile (0.1% TFA); gradient: 0–1 min: 100% A, 10 min: 50% B; 15 min: 50% B; flow: 1.0 mL/min; column: Gemini-NX C18 250×4.6 mm (Phenomenex); R_t (min): IV-14 (10.4), IV-14 MP (8.0); detection UV: λ = 254 nm and then purified under the same conditions. The solution was evaporated to dryness under reduced pressure (bath temperature < 20°C), the residue was dissolved in 5 μM NH_4HCO_3 to give a solution of IV-14 MP which was used for HPLC experiments. Purity: 96% (HPLC). MS (ESI): negative mode m/z = 496.9 ($[M + Na – 2 H]^-$), 474.9 ($[M – H]^-$); ESI HRMS: calculated for $C_{11}H_{13}IN_2O_9P^-$: 474.94088; found: 474.94127; UV: λ_{max} = 211, 261 nm.



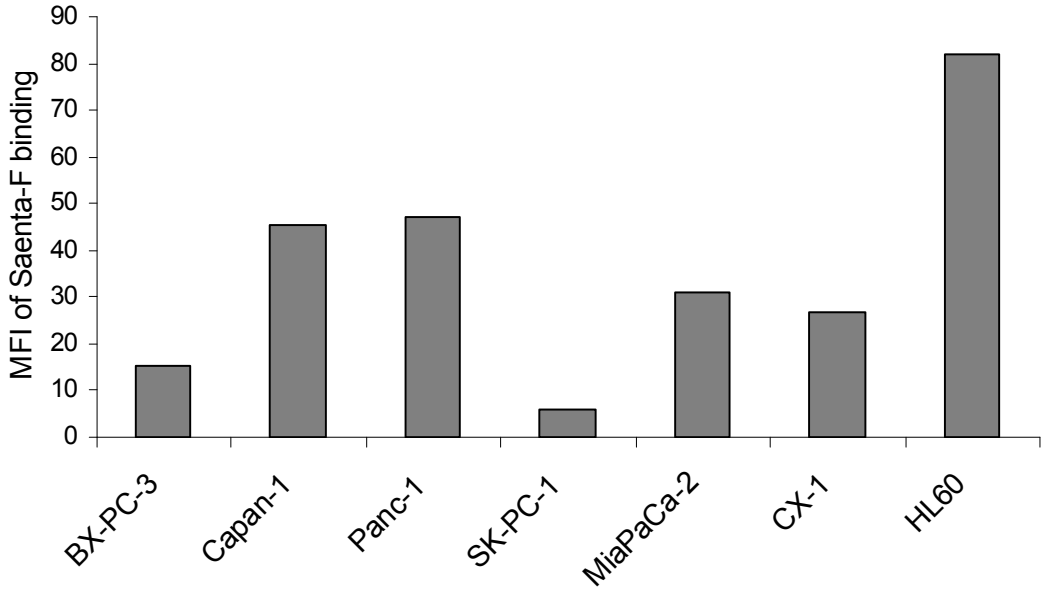
Supplemental Figure 1. Comparison of M-1 and non-labelled IV-14 monophosphate (IV-14 MP) by HPLC: (A) Non-labelled IV-14 and IV-14 MP. (B) Chromatogram of the cell lysate of Mia-PaCa-2 cells after incubation with ^{131}I-IV-14 (5×10^5 cells, 1 MBq, 1 h) mixed with non-labelled IV-14 and IV-14 MP; activity channel. (C) Chromatogram the same mixture as in (B); UV channel. The column effluent passed first through an UV-detector and then through a radioactivity detector. Conditions: eluent A: water (0.1% TFA), eluent B: acetonitrile (0.1% TFA); gradient: 0–1 min: 100 % A, 10 min 50 % B; 10–15 min 50 % B); flow: 1 mL/min; UV: λ = 254 nm; column: Nucleosil C18 HD 250×4.6 mm (Macherey-Nagel).



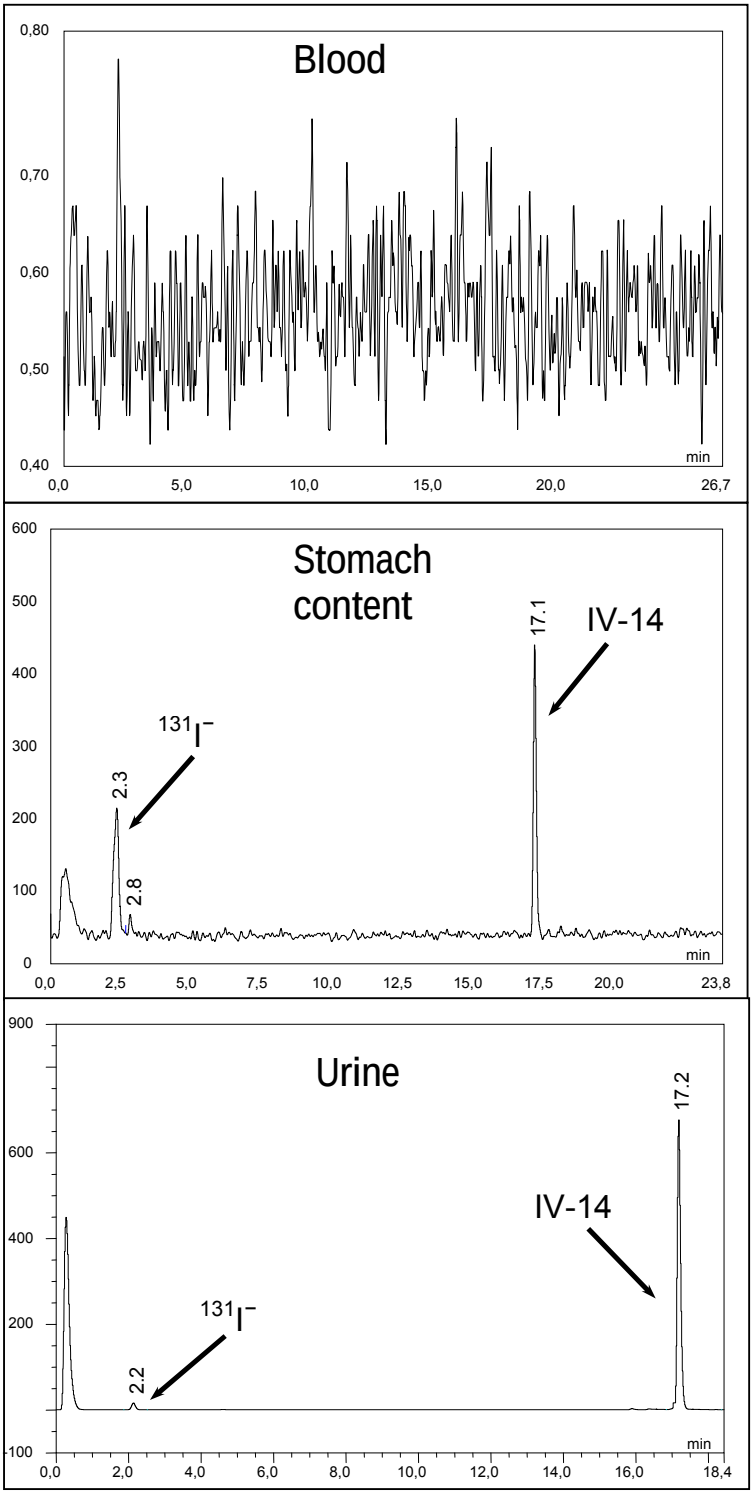
Supplemental Figure 2. Stability in serum of ^{131}I-IV-14 and ^{131}I-2'-deoxy-5-iodouridine (IdUrd).



Supplemental Figure 3. Susceptibility of IV-14 and IdUrd to cleavage by human thymidine phosphatase.



Supplemental Figure 4. $hENT_1$ transporter sites on tumor cells measured by flow cytometry with 5-(SAENTA-x8)-fluorescein. MFI – maximal fluorescence intensity.



Supplemental Figure 5. Analysis of metabolites of ^{131}I-IV-14 (4 p. i.) in HL60 xenografted SCID mice (blood, stomach content, urine). Conditions: eluent A: water (0.1% TFA), eluent B: acetonitrile (0.1% TFA); gradient: 0–12 min: 100% A, 20 min: 100% B; flow: 1.8 mL/min; column: Gemini-NX C18 250×4.6 mm (Phenomenex).