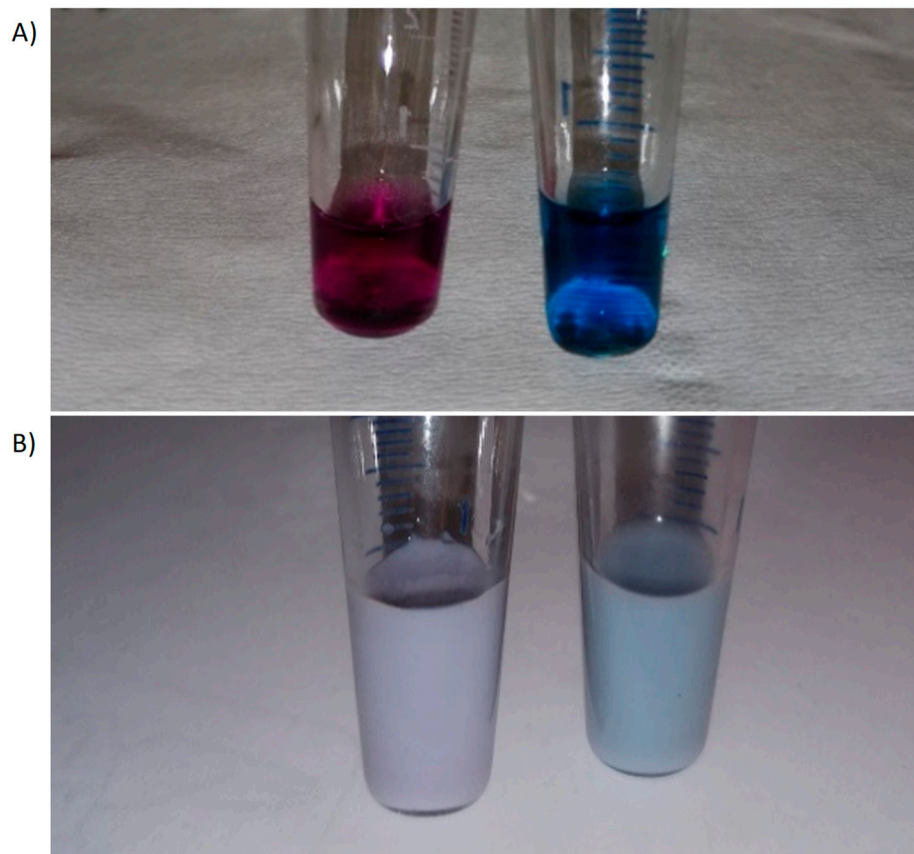


# **Alendronate-Grafted Nanoemulsions for Bone-Targeted Vincristine Delivery: Preliminary Studies on Cell and Animal Models**

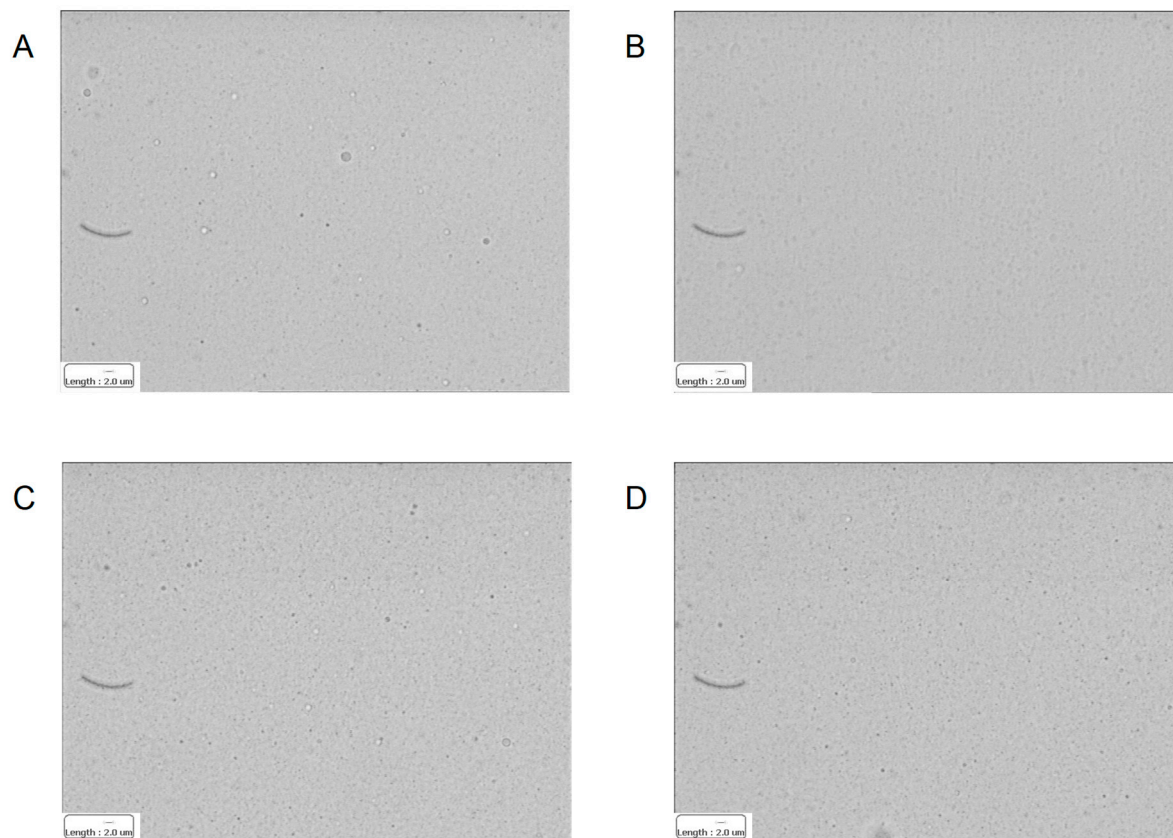
**Ian Stoppa, Chiara Dianzani, Nausicaa Clemente, Annalisa Bozza, Valentina Bordano, Sara Garelli, Luigi Cangemi, Umberto Dianzani and Luigi Battaglia**

## **Supplementary materials**

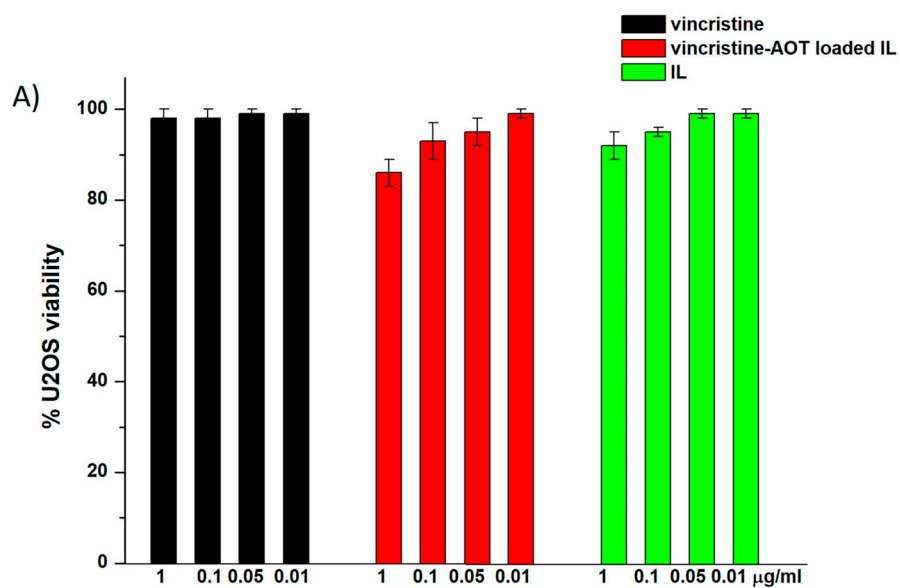
<b>Figure S1</b>	<b>Page 2</b>
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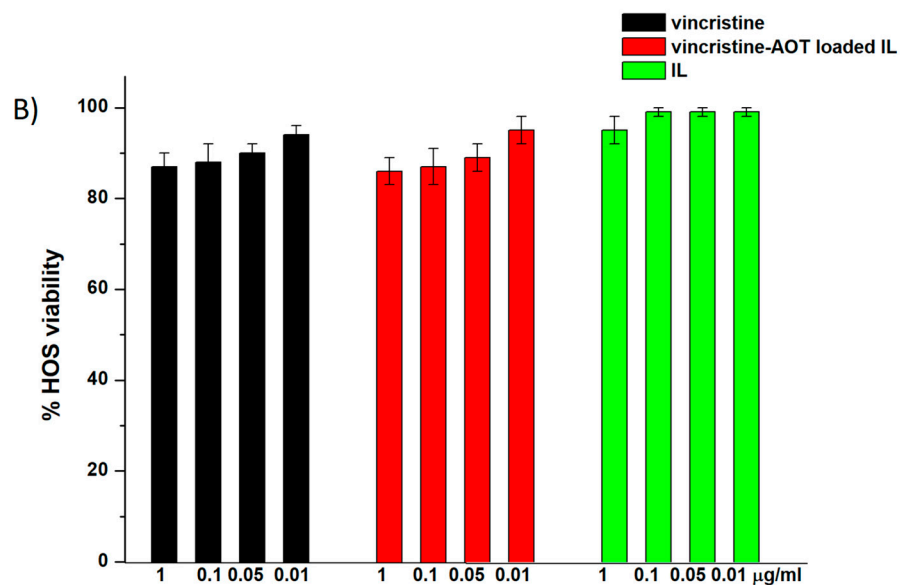
**Figure S1.** Colour change of NET, in the presence (left) and in the absence (right) of  $\text{Ca}^{2+}$ , in solution (A) and in IL (B). Abbreviations: IL: Intralipid® 10%; NET: eriochrome black T.



**Figure S2.** Optical microscopy of engineered formulations. A) blank IL; B) ALD-grafted IL; C) vincristine-AOT loaded IL; D) ALD-grafted vincristine-AOT loaded IL. Abbreviations: ALD: alendronate; AOT: sodium docusate; IL: Intralipid® 10%.

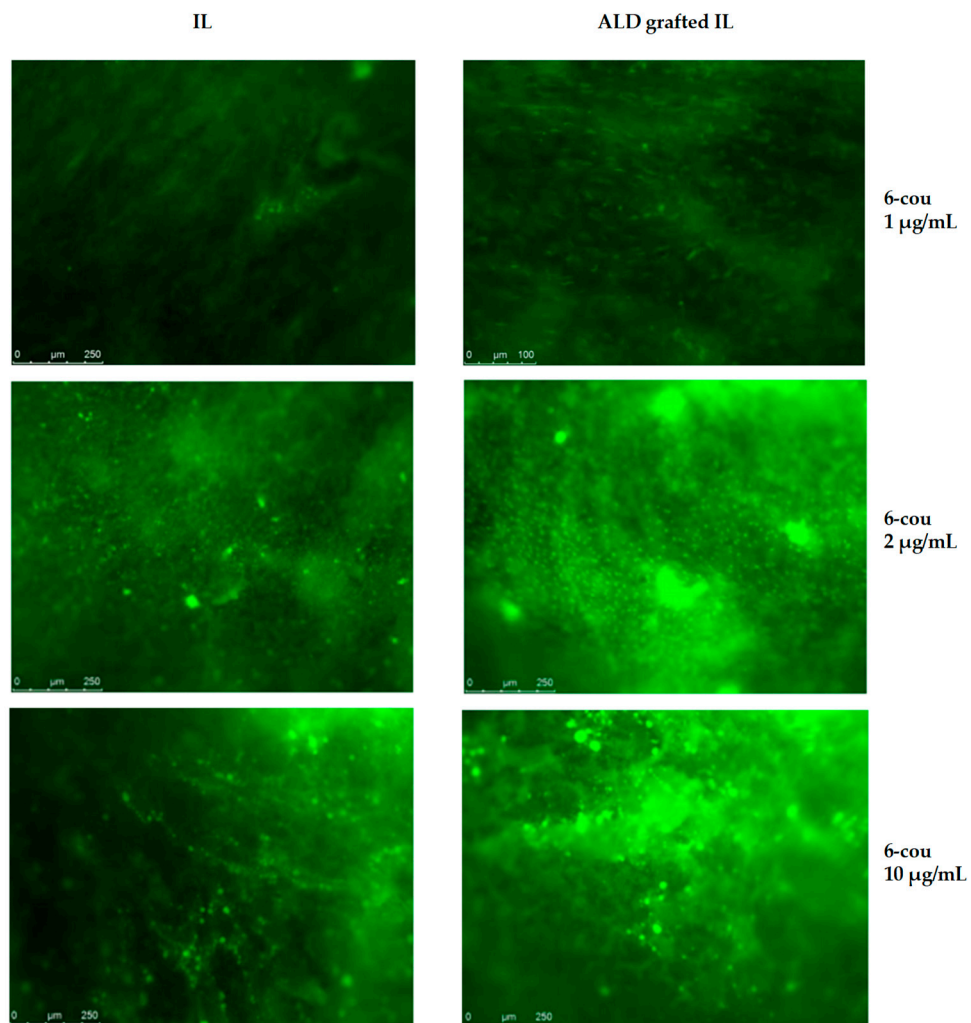


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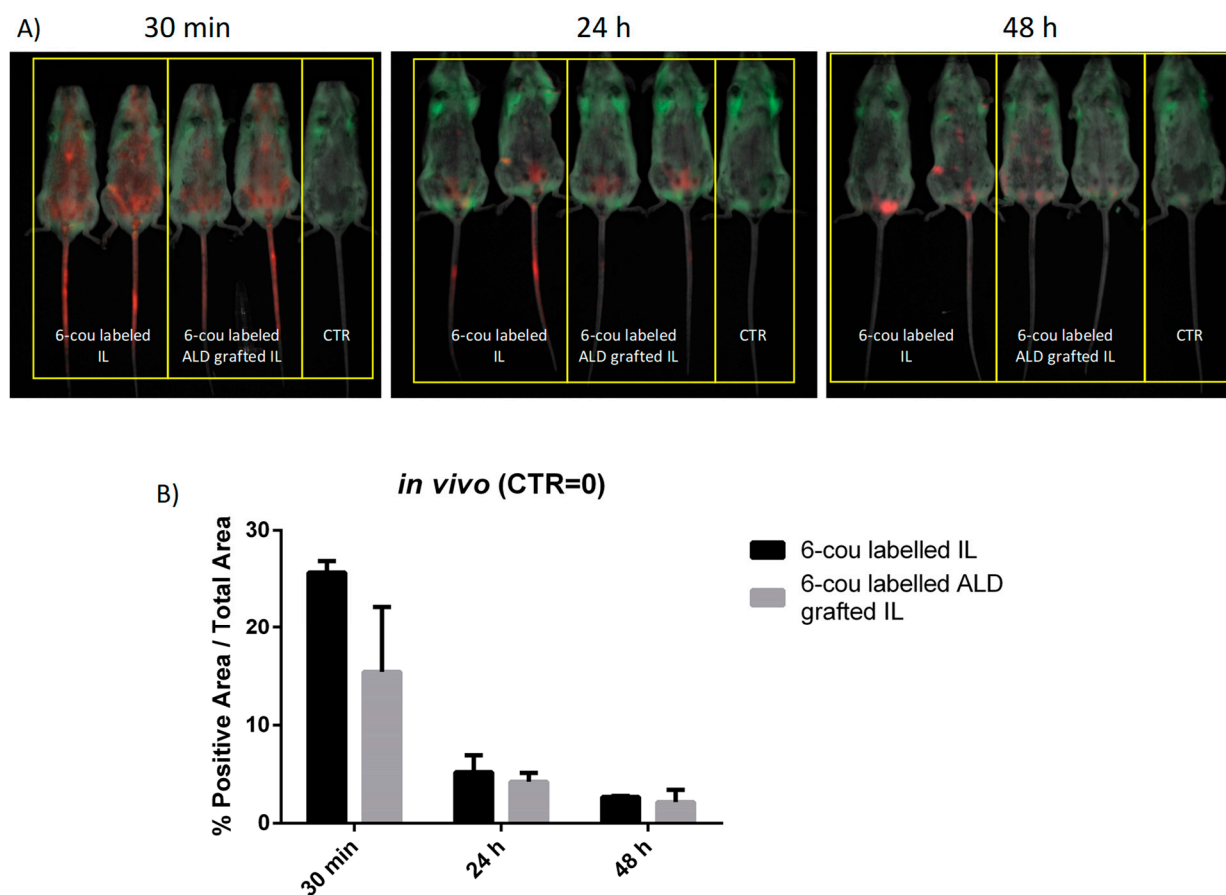


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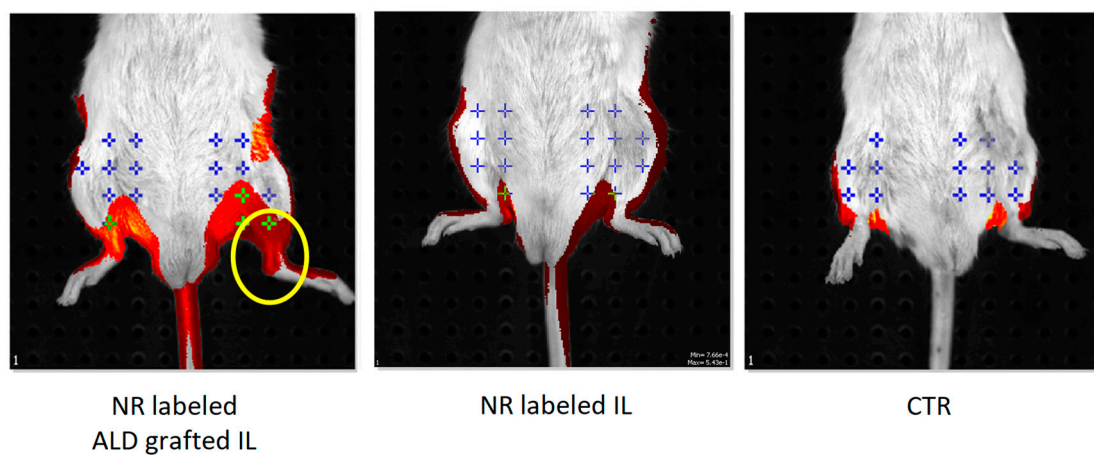
**Figure S3.** Crystal violet assay on U2OS and HOS cells after 6 hours of treatment with vincristine-based formulations. Abbreviations: AOT: sodium docusate; IL: Intralipid® 10%. A) U2OS; B) HOS.



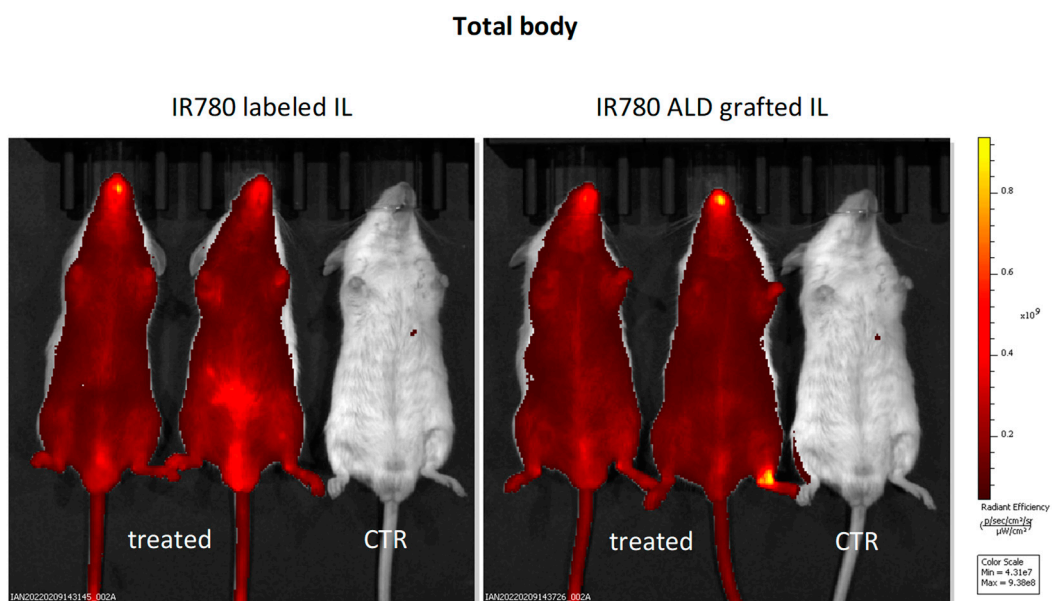
**Figure S4.** Fluorescence optical microscopy of mouse scapulae after incubation with different amounts of 6-cou-labelled nanoemulsions. Left panel: 6-cou labelled IL; right panel: 6-cou labelled ALD grafted IL. Upper panel: 1 µg/mL 6-cou; 2 µg/mL 6-cou; 10 µg/mL 6-cou. Abbreviations: 6-cou: 6-coumarin; ALD: alendronate sodium; IL: Intralipid® 10%.



**Figure S5.** *In-vivo* pharmacokinetics using IVIS (spectral unmixing mode), after administration of 6-cou-labelled nanoemulsions (n=2). A) acquisitions; B) quantification. Abbreviations: 6-cou: 6-coumarin; ALD: alendronate sodium; CTR: control; IL: Intralipid® 10%.



**Figure S6.** *In-vivo* biodistribution in mouse legs using IVIS (trans-luminescence mode), 24 hours after administration of NR-labelled nanoemulsions. Abbreviations: ALD: alendronate sodium; CTR: control; IL: Intralipid® 10%; NR: Nile Red.



**Figure S7.** *In-vivo* biodistribution (total body) of IR780-SDS-labelled nanoemulsions, 30 minutes after administration. Abbreviations: ALD: alendronate sodium; CTR: control; IL: Intralipid® 10%; IR780: 2-[2-[2-Chloro-3-[(1,3-dihydro-3,3-dimethyl-1-propyl-2H-indol-2-ylidene)ethylidene]-1-cyclohexen-1-yl]ethenyl]-3,3-dimethyl-1-propylindolium; SDS: sodium dodecyl sulfate.