Multimodal hybrid and theranostic nanoparticles as smart Trojan horses to fight cancer

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Cancer, together with circulatory diseases, is one of the primary causes of mortality worldwide and its incidence is expected to rise by about 70 per cent (from 14 million to 22 million new cases/year) over the next two decades. There is a continuous growth of scientific studies on the genetic and molecular bases of this severe disease on one side and on the development of new smart and nano-sized materials as theranostic (i.e. therapeutic and diagnostic) platforms on the other.

Several kinds of existing nanomedicine tools show smart characteristics, like controlled drug delivery, cancer cell targeting ability and biocompatibility. However, challenges like their immunogenicity, stability in biological media, off-target drug delivery and pharmacokinetics are still under investigation—making these nanomedicines incomplete for clinical translation.

The ERC Starting Grant project TrojaNanoHorse (TNH) developed by Prof. Valentina Cauda aims to close the gap between existing nanomedicine tools and the clinical requirements. The team strives to develop a non-immunogenic, hybrid and stimuli-responsive nanosystem against cancer.

The TNH is made up of an inorganic nanocrystalline core with multifunctional features, such as bioimaging capabilities, stimuli-responsive action for therapy, and biodegradation. In particular, the TNH is programmed to kill the tumour cells without the use of potentially toxic drugs, i.e. chemotherapeutics. Furthermore, the TNH has a biomimetic lipid bilayer shell autologously derived from the patients, thus rendering the whole nanoconstruct fully biomimetic, haemocompatible (thus injectable) and naturally non-immunogenic. The idea is that of the Greek myth of the Trojan horse, which is not recognised as dangerous, hence is taken up inside the city, i.e. the tumour. The TNH is then completed by targeting ligands in order to be safely and precisely directed to the tumour cell.

The project, now at the fourth year from its start dated on March 2016, is achieving its three main objectives, aiming to: (1) construct the TNH and proof the therapeutic capability and targeting action; (2) assess the biodegradation and safety of the nanoconstruct; and (3) proof the bioimaging and diagnostic capabilities in a multimodal way, thus rendering the whole TNH a complete theranostic tool.

The scientific results impressively confirmed the high-risk hypotheses at the base of the project and, thanks to the top-level and multidisciplinary competences of the research team, its feasibility.

The first research efforts were devoted to developing the chemical synthesis of round-shaped, smooth-edged nanocrystals made of zinc oxide (ZnO) with a diameter of 20nm (Figure 1a). A novel method (Garino et al., 2019, V. Cauda, 2017) was set on a wet-chemical, solvo-thermal synthesis assisted by microwave, leading to highly reproducible ZnO nanocrystals in terms of size distribution, morphology and surface chemistry, biocompatibility, and low cellular toxicity, with outstanding and long-term colloidal stability in ethanol and water media.

The team also proceeded to the extraction and characterisation of the extracellular vesicles (EVs) derived from different cell lines, in particular from both cancer and healthy cells cultured in vitro (Figure 1b). Following the thorough characterisation of their properties and protein signatures, the team implemented methods for the re-engineering of the extracted EVs, to functionalise their surface with ligands for successful cancer cell targeting (Susa et al., 2019). The TNH construction was then finalised, first testing different physical and chemical parameters and coupling procedures between the ZnO NCs and the EVs (Figure 1c) (Dumontel et al., 2019).

The various techniques used to characterise the TNH formation reported

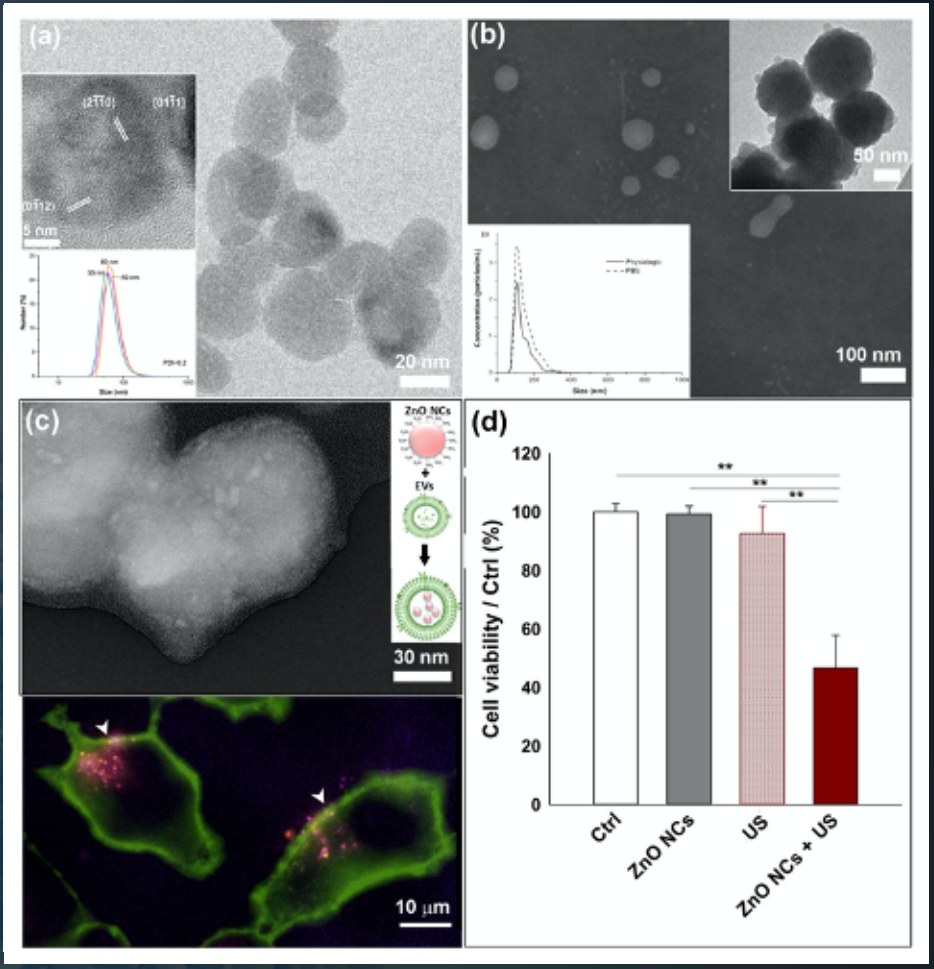
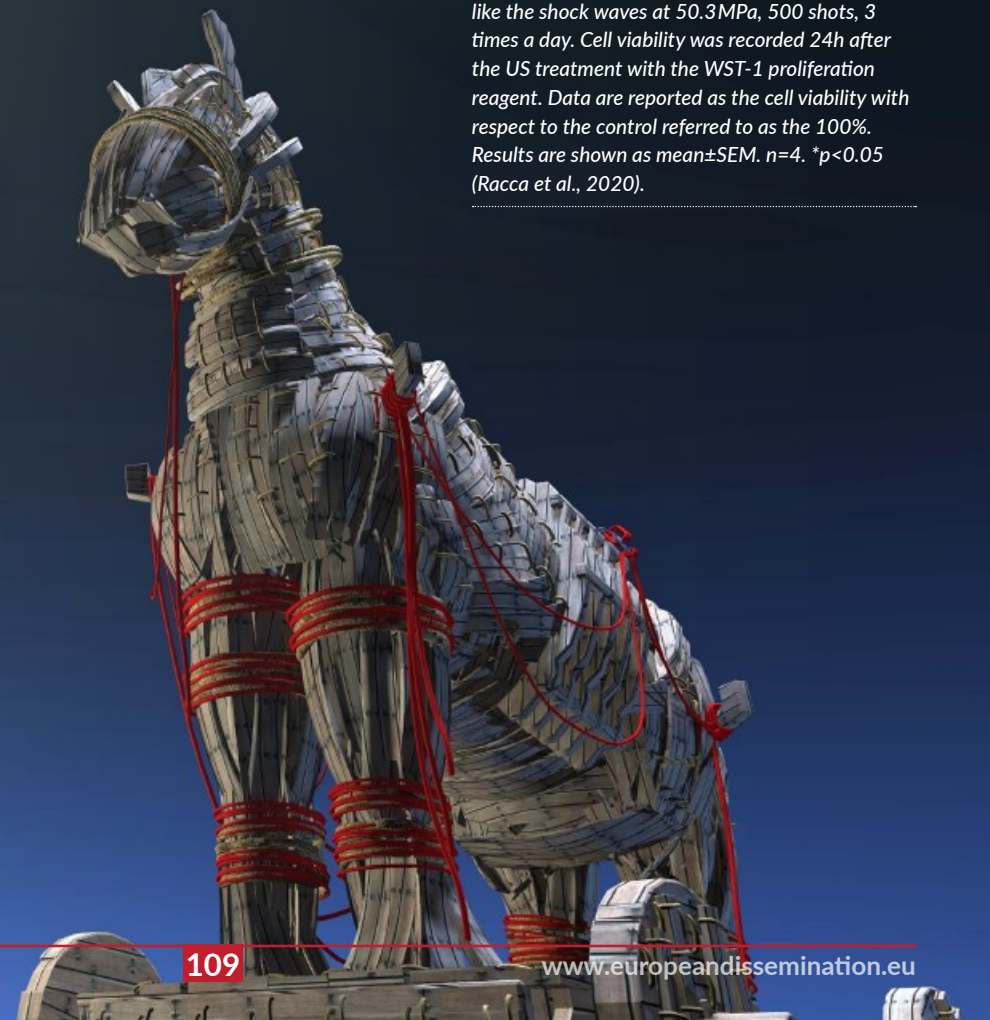


*Figure 1: (a) Conventional and high-resolution transmission electron microscopy (CTEM and HRTEM, this last in the inset) images of ZnO NCs obtained via microwave-assisted synthesis; DLS (Dynamic Light Scattering) in EtOH of the ZnO NCs (inset) (Garino et al., 2019). (b) Field emission scanning electron microscopy (FESEM) and TEM (in the inset) of the extracellular vesicles extracted from KB adenocarcinoma cancer cells and their size distribution measured by Nanoparticle-tracking analysis in phosphate-buffered saline (PBS) and physiologic solutions (NaCl 0.9%wt in water) showing the narrow size distribution centred at 110nm (Dumontel et al., 2019). (c) TEM image at 80kV of the TrojaNanoHorse freshly prepared without any further fixing or staining process with inverted colours to improve the zinc oxide nanocrystal visibility. Inset shows a scheme of the coupling process to build the TNH. Below the cellular uptake of TNH by KB cancer cells is presented. The cell membranes are depicted in green, extracellular vesicles in orange and zinc oxide nanocrystals in purple. The arrow highlights the colocalisation between zinc oxide and extracellular vesicles with the cell membrane during internalisation (Dumontel et al., 2019). (d) Synergistic effect of ZnO NCs and ultrasound in killing cancer cells. Four different samples were prepared: control untreated cells KB adenocarcinoma (Ctrl), cells only incubated with 10µg/mL ZnO NCs for 24h (ZnO NCs); cells treated with ultrasound (US) and cells incubated with NCs and treated with US (ZnO NCs + US). Cells were treated with a particular source of US, like the shock waves at 50.3MPa, 500 shots, 3 times a day. Cell viability was recorded 24h after the US treatment with the WST-1 proliferation reagent. Data are reported as the cell viability with respect to the control referred to as the 100%. Results are shown as mean±SEM. n=4. *p<0.05 (Racca et al., 2020).*

that the coupling process is a consequence of various mechanisms, including thermodynamic, kinetic and electrostatic ones. The team is still working to increase the yield of the final TNH for future in vivo tests. However, solid proof of colloidal biostability and concentration-dependent cytocompatibility have been proved; results showed the importance of masking the nanocrystals prior to contact with living systems, to obtain an improved biostability, controlled biodegradation over time and enhanced cell internalisation (Dumontel et al., 2017; Limongi et al., 2019). Finally, the haemocompatibility tests of the pristine ZnO nanocrystals have successfully shown no coagulation activity in human plasma.

Concerning the stimuli-responsive therapy, the team developed an unconventional and safe methodology for nanocrystal remote activation with ultrasound stimulation to produce damage to cancer cells and



lead highly toxic species (V. Cauda, 2018), first in acellular media (Vighetto et al., 2019; Ancona et al., 2020) and then with cancer cells (Racca et al., 2020). The team strove to fully understand the different key parameters to control the stimulation, the nanocrystals activation and their complex biological effects on cancer cells (Canavese et al., 2018). Strikingly, a strong anti-proliferative effect was observed only upon the synergistic combination of ZnO nanocrystals and multiple ultrasound treatments in in vitro cancer cells while no effect on cell vitality was detected when the cells are treated separately with the nanocrystals or the ultrasound stimulation only (Racca et al., 2020) (Figure 1d). These results suggested the existence of a powerful synergy, which is now opening the way to in vivo tests.

Based on the strong expertise gained to set up the stimuli-responsive therapy and related equipment, advancements were also made on the bioimaging side using the activation stimuli, to exploit a novel, unconventional imaging procedure based on echographic contrast imaging (Ancona et al., 2020). We can thus proudly state that the TNH is accomplishing its role as multimodal and novel theranostic biomimetic nanodevice, towards a never before reported novel clinical translation.

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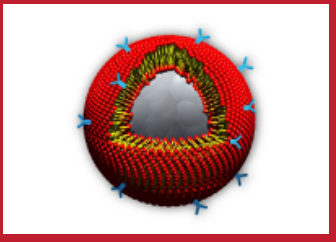

PROJECT SUMMARY

The multidisciplinary ERC Starting Grant project "Hybrid immune-eluding nanocrystals as smart and active theranostic weapons against cancer" (TrojaNanoHorse) aims to develop a new generation of multifunctional theranostic nanosystems displaying non-immunogenicity, improved cancer treatment, cell imaging, and high safety for the hosting organism.

PROJECT LEAD PROFILE

Born in Turin, Prof. Cauda received her PhD in Material Science and Technology from Politecnico di Torino University in 2008. After spending three years Postdoc at LMU Munich (Germany) and five years at IIT (Italy), she received her ERC Starting Grant in 2015 to create novel hybrid and biomimicking nanomaterials. Prof. Cauda has authored over 100 peer-reviewed articles and four international patents.

PROJECT PARTNER

The TrojaNanoHorse project is based at Politecnico di Torino (Italy) taking advantage of its state-of-the-art nanomaterial laboratories and knowledges. Collaboration partners include groups from the University of Turin, and from other EU countries, like Germany, UK, Spain, and beyond.

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FUNDING

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme under grant agreement no. 678151.