Functionalized chitosan nanobubbles as a strategy to reverse immunosuppressive tumor microenvironment

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INTRODUCTION

Chitosan nanobubbles (NBs) have shown a great potential as nanoplatform for anticancer drug delivery. They represent a promising strategy to overcome the limitations of anticancer drugs, allowing site-specific delivery, decreased side effects and drug resistance [1]. Interestingly, the polymer shell can be functionalized with specific ligands for an active targeted delivery.

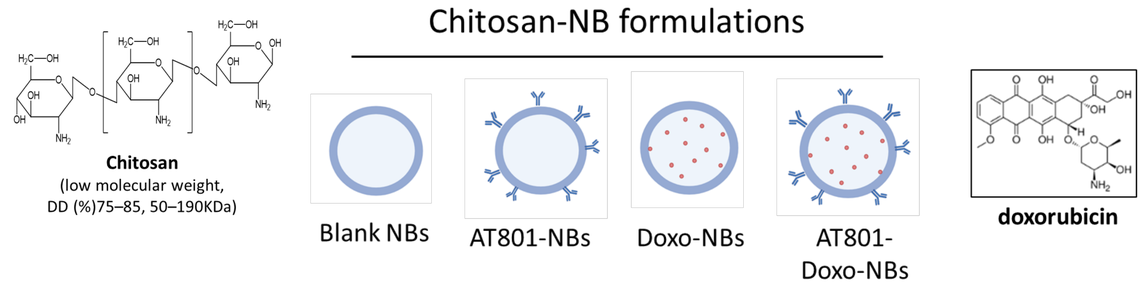
CD138, also known as Syndecan-1, may be a useful immunotherapeutic target for multiple myeloma (MM) treatment being highly expressed on MM cell surfaces, whereas it is express at low levels in benign cells. Given its structure, CD138 undergoes a shedding process by metalloproteinase-9 (MMP-9) secreted by different cells, such as M2 macrophages. That leads to interaction between shed-CD138 and different microenvironmental components by which CD138 plays a significant role in tumor growth, adhesion and invasion [2].

Moreover, due to their chitosan composition, NBs itself can modulate the immunological crosstalk within the tumor site and shift various immune cell types, such as macrophages, towards an inflammatory phenotype, reversing the immunosuppressive tumor microenvironment [3].

AIM OF THE WORK: to investigate the cytotoxic effects and capability to affect tumor microenvironment of chitosan NBs conjugated with the anti-CD138 antibody and loaded with doxorubicin for multiple myeloma treatment.

EXPERIMENTAL METHODS

- Formulation of chitosan NBs loaded with **doxorubicin** and conjugated with **AT801**, a mouse monoclonal antibody (mAb) of the IgG class directed against a CD138 epitope in close proximity to the cell membrane (patent n. 102025000010005, Ministero delle Imprese e del Made in Italy)
- Fluorescent-labelled NBs prepared binding cyanine 5.5 (Cy5.5) to the shell of NBs

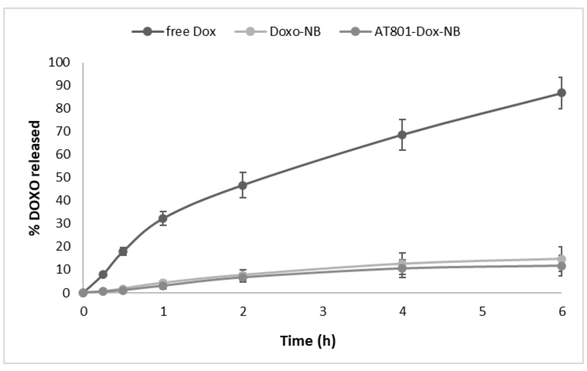


- *In vitro* characterization (morphology, physico-chemical parameters, drug loading, *in vitro* release studies), *in vitro* cytotoxicity evaluation, *in vivo* biodistribution studies, *ex vivo* microenvironmental investigation and *in vitro* evaluation of NB effects on mouse macrophages

RESULTS

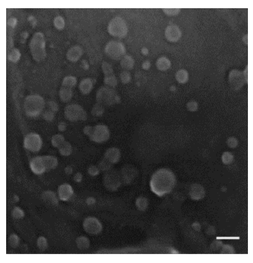
In vitro characterization of NB formulations

	Average diameters ± SD (nm)	Polydispersity index ± SD	Zeta potential ± SD (mV)	Doxo concentration (μg/ml)	Antibody concentration (μg/ml)
Blank NB	325.4 ± 10.5	0.20 ± 0.02	31.14 ± 1.40	-	-
AT801-NB	320.7 ± 10.3	0.22 ± 0.01	28.60 ± 1.33	-	100
Doxo-NB	342.5 ± 8.6	0.21 ± 0.02	30.86 ± 1.56	200	-
AT801-Doxo-NB	340.2 ± 11.4	0.22 ± 0.02	27.95 ± 2.07	200	100
Cy5.5-NB	326.8 ± 9.5	0.22 ± 0.02	29.44 ± 1.22	-	-
AT801-cy5.5-NB	322.3 ± 12.1	0.21 ± 0.01	26.80 ± 1.18	-	100



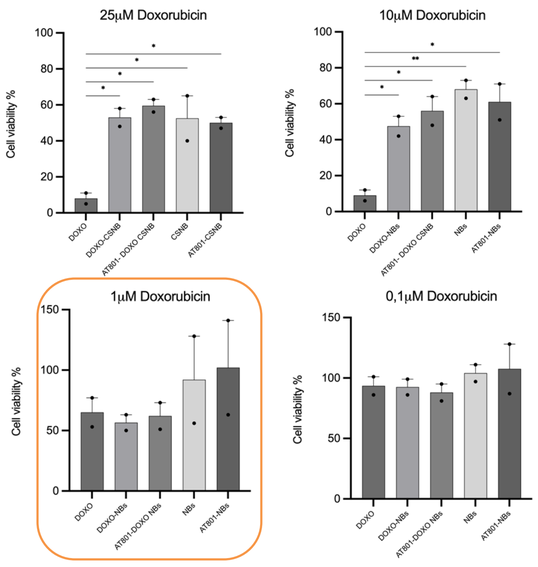
In vitro release kinetics of Dox from targeted or untargeted NBs

FESEM image of chitosan nanobubbles (scale bar 400 nm)



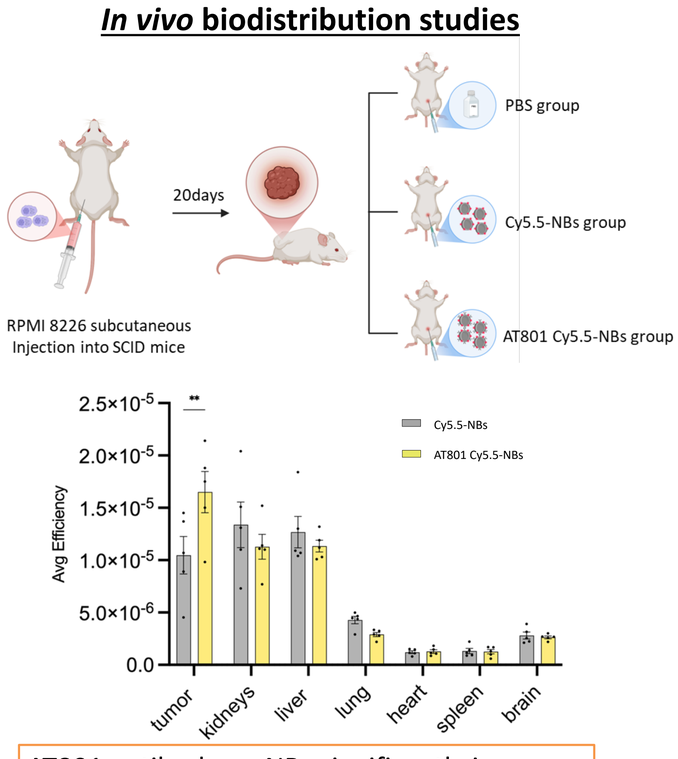
DRUG LOADING: 7.42%
ENCAPSULATION EFFICIENCY: 90.50%

In vitro cytotoxicity of NB formulations



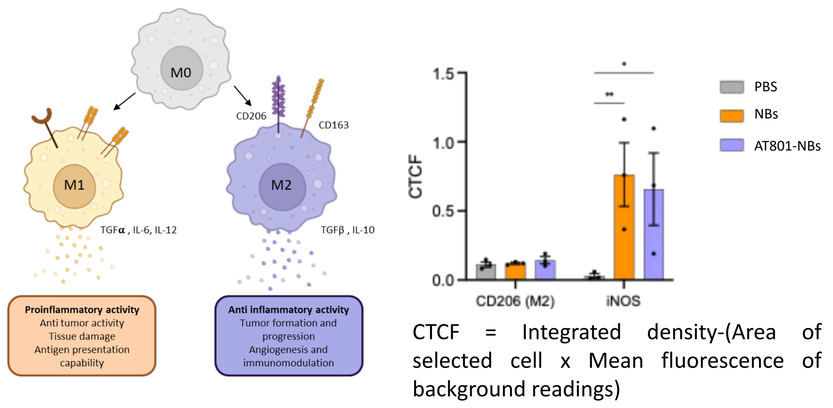
At a concentration of 1μM, Dox-loaded NBs induced 50% cell killing compared to cells treated with empty NBs, both in non-targeted and targeted systems, which showed 100% cell viability.

In vivo biodistribution studies

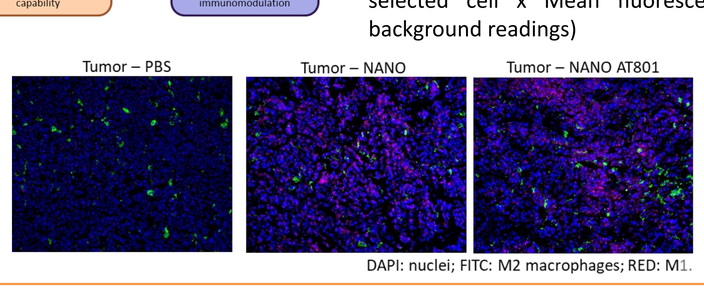


AT801 antibody on NBs significantly increases the amount of NBs reaching the tumor site

Ex-vivo microenvironmental investigation



CTCF = Integrated density-(Area of selected cell x Mean fluorescence of background readings)

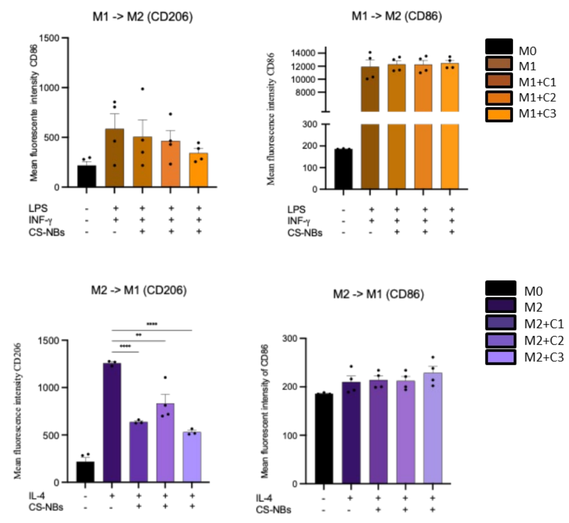


DAPI: nuclei; FITC: M2 macrophages; RED: M1.

No differences are visible in the recruitment of M2 macrophages between the three compared masses. The amount of M1 is higher in the mass treated with NBs alone compared to PBS and is even higher in the mass treated with AT801-NBs.

In vitro effects of NBs on mouse macrophages (RAW 264.7)

RAW 264.7 (M0) polarized for 48h with LPS+INFγ in M1 and with IL-4 in M2. After that, cells were washed and treated for 24h with increase amount on unconjugated chitosan NBs.



MFI of M1 and M2 specific markers on unstimulated and stimulated with different concentration of chitosan NBs for 24h

No differences are visible in M1 when treated with increased amounts of chitosan NBs but on M2 macrophages a reduction in CD206 fluorescent intensity was observed as well as an increase in CD86 fluorescent intensity, directly linked to the amount of NBs

CONCLUSION

Stable nanosuspension of NBs conjugated with anti-CD138 antibody AT801 showing average diameters of about 300 nm and low polydispersity index was obtained. The NBs were able to load doxorubicin with good encapsulation efficiency and release it with prolonged release kinetics. *In vivo* biodistribution studies showed that the NBs can accumulate in the tumor and that their conjugation with AT801 significantly increased the amount of NBs reaching the tumor mass. No differences in the recruitment of M2 macrophages but an increase of M1 population in the tumor masses of NBs treated mice were observed. Results suggested that chitosan NBs could polarize M2 macrophages toward M1 phenotype. Chitosan nanobubbles conjugated with the anti-CD138 antibody might represent a promising nanoplatform for anticancer drug delivery for multiple myeloma treatment.

REFERENCES: [1] Mossenta, M.; Argenziano, M.; Capolla, S.; Busato, D.; Durigutto, P.; Mangogna, A.; Polano, M.; Sblattero, D.; Cavalli, R.; Macor, P.; Toffoli, G.; Dal Bo, M. Idarubicin-loaded chitosan nanobubbles to improve survival and decrease drug side effects in hepatocellular carcinoma. Nanomedicine (London, England), 20(3), 255–270 (2025). [2] Riccardi F, Tangredi C, Dal Bo M and Toffoli G; Targeted therapy for multiple myeloma: an overview on CD138-based strategies. Front. Oncol. (2024) 14:1370854. doi: 10.3389/fonc.2024.1370854 [3] Ding et al, Recent Advances in Chitosan and its Derivatives in Cancer Treatment, Front Pharmacol. 2022 Apr 28;13:888740. doi: 10.3389/fphar.2022.888740