Glypican-1 targeted nanobubbles as an advanced nanomedicine for pancreatic adenocarcinoma treatment

Argenziano M.[1], Busato D.[2,3], Capolla S.[3], Toffoli G[3], Sblattero D.[2], Macor P.[2], Dal Bo M.[3], Cavalli R.[1]

[1] University of Torino, Department of Drug Science and Technology, Turin, Italy

[2] University of Trieste, Department of Life Sciences, Trieste, Italy

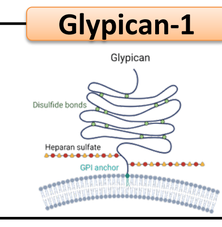
[3] Aviano Oncological Center, Experimental and Clinical Pharmacology Unit, IRCCS, Aviano, Italy

monica.argenziano@unito.it

INTRODUCTION

Systemic chemotherapeutic agents show limited efficacy in pancreatic ductal adenocarcinoma (PDAC), due to poor tumor penetration, intrinsic resistance mechanisms, and the occurrence of off-target toxicity [1]. To address these limitations, antibody-conjugated nanoplatforms have emerged as a promising strategy to selectively target PDAC cells. Among potential targets, the proteoglycan Glypican-1 (GPC1) is particularly attractive because it is highly and specifically expressed on the surface of PDAC cells [2]. Chitosan shelled nanobubbles (NBs) have demonstrated strong potential for anticancer drug delivery and their polymer shell can be easily functionalized with specific ligands for an active targeted delivery [3]. **AIM OF THE WORK** → development of doxorubicin-loaded AT101 mAb-conjugated chitosan NBs as targeted nanomedicine for PDAC treatment.

Glypican-1



- 62kDa cell surface proteoglycan
- GPI anchor covalently bound to the C-terminus and this is crucial for the association with other protein on cell membrane

IgM antibody (**AT101**), specifically recognizing GPC1

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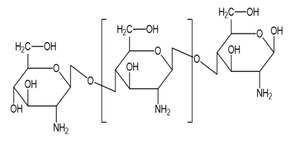
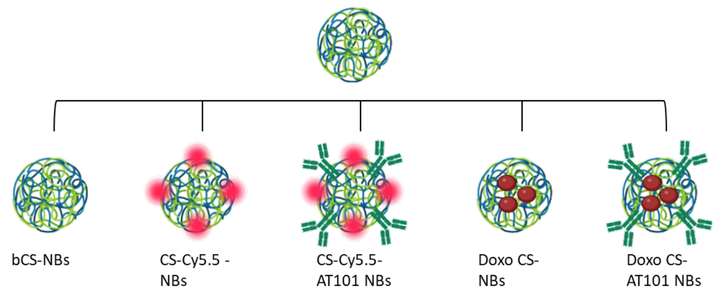
- Target specificity in FC, IFF on cells and on tissues
- Target ability in xenograft model mice
- *In vivo* therapeutic effects
- Efficiently delivery NBs in tumor

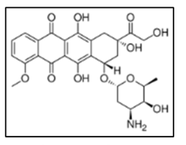
➢ Formulation of chitosan NBs loaded with doxorubicin and conjugated with AT101 mAb

➢ Fluorescent-labelled NBs prepared binding cyanine 5.5 (Cy5.5) to the shell of NBs



Chitosan
(low molecular weight, DD (%)75–85, 50–190KDa)





doxorubicin

➢ *In vitro* characterization (morphology, physico-chemical parameters, drug loading, *in vitro* release, stability), *in vitro* cytotoxicity evaluation, *in vivo* and *ex vivo* studies on BXPC3 xenograft mouse model (biodistribution studies, evaluation of NB therapeutic effect and toxicity)

RESULTS

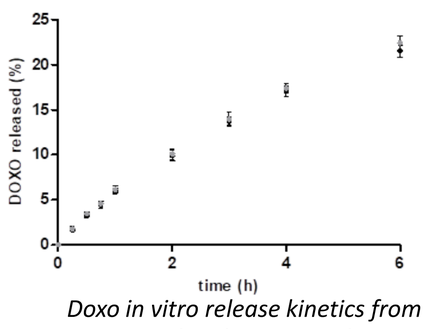
➢ *In vitro* physico-chemical characterization

Physico-chemical characteristics of NB formulations

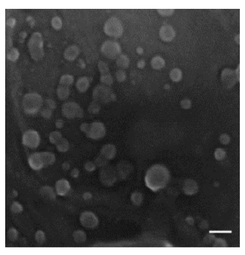
Sample	Average diameter ± SD (nm)	PDI	Zeta potential ± SD (mV)
CS-NBs	355.6 ± 9.5	0.20	+30.50 ± 1.52
CS-NBs-Cy5.5	360.5 ± 11.2	0.22	+28.64 ± 1.85
AT101-CS-NBs-Cy5.5	358.2 ± 10.4	0.21	+25.80 ± 2.12
CS-NBs-doxo	364.8 ± 11.5	0.20	+31.02 ± 1.47
AT101-CS-NBs-doxo	363.6 ± 10.6	0.22	+26.24 ± 1.96

Concentration (NTA analysis): $1x10^{12}$ NB/ml

DRUG LOADING: 2.58 ± 0.05%
ENCAPSULATION EFFICIENCY: 91 ± 0.2%

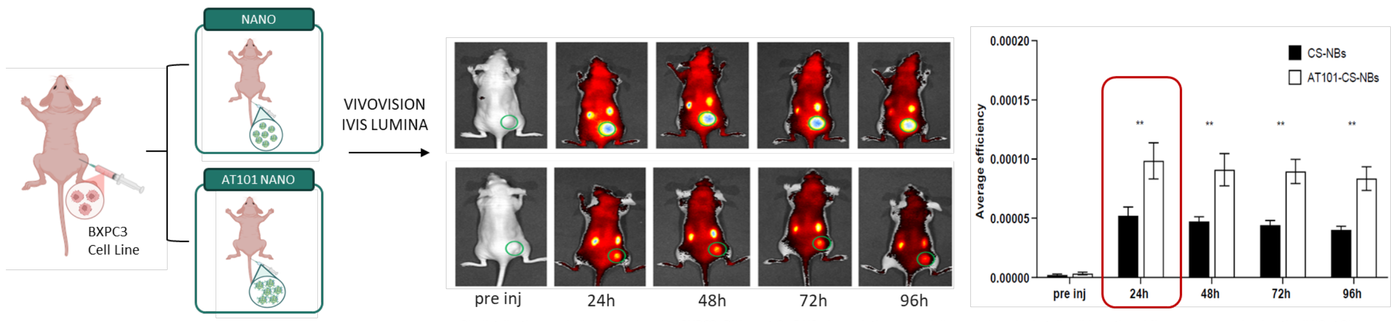


Doxo in vitro release kinetics from targeted and untargeted NBs

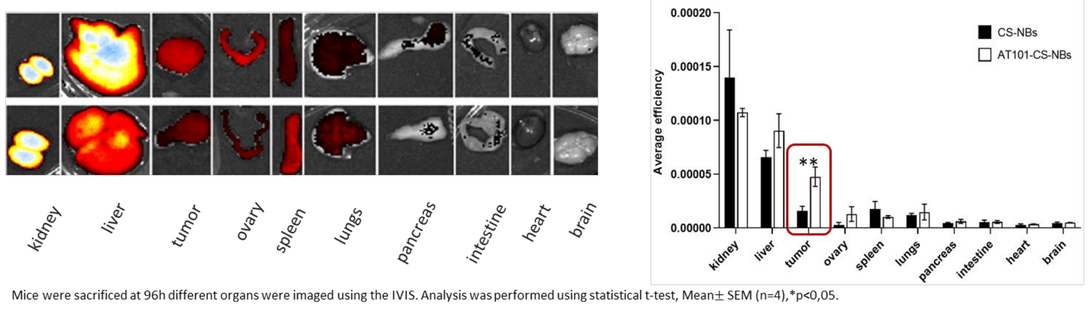


FESEM image of CS-NBs (scale bar 400 nm)

➢ *In vivo* and *ex vivo* biodistribution



Biodistribution analysis in mice bearing BXPC3. Different Cy.5 NBs formulation were delivered via a single tail vail injection. Mice were imaged using the IVIS at 0h, 24h, 48h, 72h, 96h. Analysis was performed using statistical t-test, Mean± SEM (n=4),*p<0,05.



Mice were sacrificed at 96h different organs were imaged using the IVIS. Analysis was performed using statistical t-test, Mean± SEM (n=4),*p<0,05.

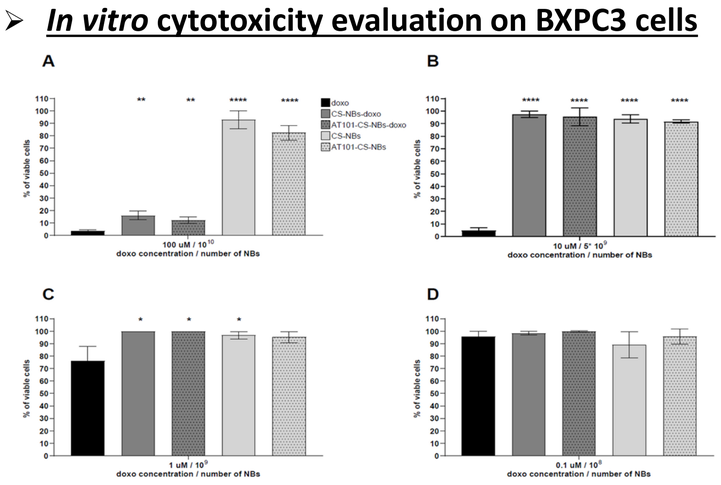
➢ Evaluation of NB toxic effects in mouse tissues

Histopathological features	Doxo	NB Doxo	NB Doxo AT101
Steatosis	+	-/+	-/+
Inflammation	-	-/+	-/+
Necrosis	-	-	-
Fibrosis	-	-	-
Cholestasis	-	-	-

Histopathological features	Doxo	NB Doxo	NB Doxo AT101
Interstitial fibrosis	-	-	-
Inflammatory infiltrate	++	-/+	-/+
Myocyte damage	-/+	-	-
Ischemic damage	-	-	-
Vascular damage	-	-	-
Thrombosis of the vessel	-	-	-

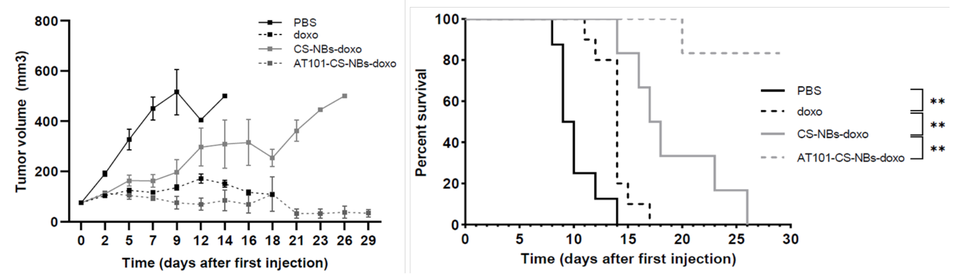
Scoring system: -, negative; +, minimal to mild; ++, moderate; +++, severe.

➢ *In vitro* cytotoxicity evaluation on BXPC3 cells



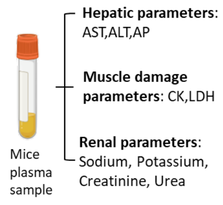
➢ *In vivo* evaluation of NB therapeutic effect

Dose: 0.67 mg/kg of doxo with an administration every other day (corresponding to a dose of 2 mg/kg of doxo in a week

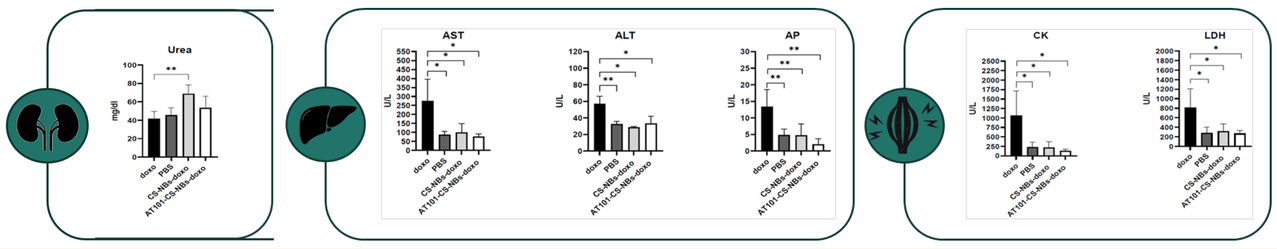


94% of animals treated with AT101-CS-NBs-doxo reached the experimental endpoint at 29 days while none of the animals treated with CS-NBs-doxo reached the endpoint.

➢ Evaluation of haematochemical parameters of toxicity



Increase in AST, ALT, AP, CK and LDH levels for systemic administration of free DOX, consistent with know hepatic, muscular and cardiac toxicity profiles. In contrast, mice treated with CS-NBs-doxo and AT101-CS-NBs-doxo show reduced levels for the same parameters, comparable with those of PBS-group. An increase in urea concentration was observed in the CS-NBs-doxo group but not in AT101-CS-NBs-doxo group. Probably, this is due to the tropism of CS for kidneys, but the presence of AT101 brought the values back into the normal range.



CONCLUSION

Stable nanosuspensions of NBs with an average diameter of about 350 nm, low polydispersity index and positive surface charge were obtained. The NBs were able to load doxo with good encapsulation efficiency (more than 90%) and release it with a slow and prolonged *in vitro* release kinetics. The blank NBs exhibited high biocompatibility and doxo retained its cytotoxic activity when loaded into the NBs. *In vivo* biodistribution studies in PDAC xenograft mice showed that the NBs can accumulate in the tumor and that their conjugation with AT101 significantly increased the amount of NBs reaching the tumor mass. Doxo-loaded NBs were able to reduce tumor growth and statistically increase the survival rate of mice compared to free doxo. The efficacy of doxo-CS-NBs was significantly enhanced by the presence of AT101 as targeting agents. The toxicity of free doxo was decrease by the loading in CS-NBs. AT101 further decrease the off-target toxicity, improving biodistribution and tumor-specific administration. Particularly, AT101 reduce renal accumulation and the resulting renal burden.

REFERENCES:

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