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ABSTRACT SUMMARY
A new delivery system was developed for the targeted treatment of solid tumors. The nano vesicles are derived from mesenchymal stem cells (MSC) plasma membrane, which is specifically recognized by the tumor microenvironment and tumor cells. In vitro and in vivo assays were conducted on a prostate cancer model to reassure that specificity and to evaluate the feasibility of the system to inhibit tumor growth.

INTRODUCTION
The ultimate goal in cancer drug-delivery is a 'magic-bullet' that provides a versatile platform for site-specific targeting of multiple cancers. Targeted therapy is aimed to affect only tumor cells and by that prevents additive adverse effects characteristic to non targeted systemic treatment. Here, we present a novel drug-delivery system based on vesicles composed from the cytoplasmatic membranes of MSC, known for their natural targeting of multiple cancers and hypo-immunogenicity. Encompassing MSC surface proteins and armed with their unique targeting capabilities, the vesicles may be loaded with an anti-cancer drug and can be selectively targeted against multiple cancers.

EXPERIMENTAL METHODS
Cell derived vesicles (CDV) were prepared as followed: human MSC (5*10^7 per sample) were harvested, washed, and suspended in hypotonic buffer. After incubation period cells were homogenized and sonicated to achieve sealed spheres of ghosts. Finally, the ghosts were extruded through polycarbonate membrane to a nano sized vesicles. In vitro and in vivo experiments were conducted on prostate cancer cell-line (PC3). Athymic nude and C57 Black mice were used for the in vivo set of experiments. The anti cancer drug used on this project is sTRAIL (the soluble form of the Tumor necrosis Related Apoptosis Inducing Ligand protein, produced in our lab).

RESULTS AND DISCUSSION
Cell derived vesicles (CDV) preparations were shown to resemble common nano-scaled drug delivery systems in size and morphology. DLS evaluation resulted in average diameter size of 180 nm. Via Cryo-TEM imaging the system could be observed, as can be seen in figure 1.

![Figure 1: MSC CDV as the new carriers for tumor-targeted delivery](image)

In vitro selectivity evaluation assay was performed; PC3 were incubated with MSC-CDV and washed. Clear preference of attachment was shown by the confocal microscopy imaging (figure 2). Selectivity was
also assured quantitatively compared to objective controls (data not shown).

**Figure 2: In vitro selective targeting evaluation.**
The binding of CDV to PC3 cells was evaluated using confocal microscopy over incubation time depicting micrographs of 3-dimensional projection. CDVs (white arrows) could be observed attached to the cells membrane and inside the cells.

Bio-distribution *in vivo* assay showed accumulation of CDV at the tumors, 24 hr and 1 week post IP administration. This specific accumulation was demonstrated for human and rat MSC CDV (figure 3). TRAIL loaded human MSC CDV were IP injected to PC3 tumor bearing mice. Mice were monitored for 2 weeks. Significant inhibition in tumor growth was achieved by TRAIL loaded CDV, but not for other control groups (figure 4).

**Figure 3: In vivo targeting of CDV toward PC3 tumor.**
Athymic nude mice (n=8) were inoculated with human prostate cancer cells (PC3). MSC CDV targeting ability was evaluated *in vivo* and specific accumulation at the tumor is observed for the human MSC CDV, but not for smooth muscle cell (SMC= primary human cells) derived vesicles.

**Figure 4: MSC CDV as the new carriers for tumor-targeted delivery.** TRAIL was entrapped in the MSC CDV and the drug delivery system was evaluated *in vivo* (n=8; bars are +/- s.e.m; ** in t test analysis- p value<0.01).

Another set of *in vivo* assays was performed to assess CDV immunogenicity and cytotoxicity. No significant differences between mice treated with CDV and control (un-treated mice) could be observed on RT real time PCR analysis for pro-inflammatory cytokines. H&E of liver samples showed no significant effect as a result of CDV IP injection (data not shown).

**CONCLUSION**
MSC CDV drug delivery system is a new alternative for the treatment of solid tumors that naturally recruit MSC. So far, findings regarding the ability of the CDV to target and to inhibit tumor growth and together with the low immunogenicity pattern of the system, seemed promising, though further investigation is required.

**REFERENCES**

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