

# Abstract

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Cancer is an extremely complex disease characterized by genetic instability which drives the acquisition of several capabilities that aid tumor development. The immune system plays an important part in cancer elimination; however cancer cells actively contribute to an immunosuppressive microenvironment that counteracts potent antitumor immune responses. Accordingly, there is an enormous interest in the field of cancer research to develop immunotherapeutic strategies that harness the immune system to fight cancer. Meanwhile, the recognition that the efficacy of some conventional cytotoxic chemotherapeutic agents can be attributed to mobilization of antitumor immunity has refueled the interest in these agents. Problems with anticancer agents relating to toxicity, poor pharmacokinetics and biodistribution can be addressed by the use of liposomes as drug carriers. The first chapter in this thesis gives an introduction into these presented subjects of cancer, antitumor immunity, cancer treatment, and application of liposomes for delivery of anticancer therapeutics.

In the second chapter, a liposomal drug delivery system of the cytotoxic anticancer agent mitoxantrone (MTX) is described. MTX-loaded liposomes were characterized with respect to their ability to induce immunogenic cell death and stimulate immunity. When tested in an established solid tumor model, liposomal mitoxantrone displayed remarkable antitumor effects, which was further enhanced by combination with an immune checkpoint inhibitor. A tumor microenvironment analysis of the immune infiltration and state of maturation revealed immunomodulation by liposomal MTX. Collectively, these studies highlight the therapeutic potential of applying liposome-encapsulated MTX to create an *in situ* vaccine that stimulates and modulates the tumor immune contexture.

In the third chapter a cationic liposomal drug delivery system of a toll-like receptor (TLR) 7 agonist is described. This drug delivery system displayed preferential association to monocytes once exposed to whole human blood. The following lines of experiments aimed to uncover the proteins and receptors involved in the association of PEGylated and non-PEGylated cationic liposomes to monocytes. Complement proteins were demonstrated to be involved in the association of non-PEGylated liposomes to monocytes while the PEGylated liposome association was independent of complement activation. The CD14 receptor mediated parts of the monocyte association to both liposomes. Hence, these studies served to provide insight into the mode of action of cationic liposomes once exposed to full blood.

In the fourth chapter, the liposomal drug delivery system introduced in chapter 3 is further characterized for its ability to target and activate monocytes in human blood from healthy individuals and from individuals

diagnosed with cancer that were either treatment-naïve or had received prior anticancer therapy. Cationic liposomes induced several antitumor- and pro-inflammatory cytokines thus substantiating effective TLR7 agonist delivery to the endosomal target site. The cytokine profile generated in response to liposomal TLR7 agonist relative to agonist administered as a free drug suggests a therapeutic benefit of TLR agonist delivery in the context of liposomes. Furthermore, the studies provide proof-of-concept for the application of this drug delivery system for treatment of lung, melanoma and breast cancer in combination with immunogenic cell death-inducing therapy or checkpoint inhibitors.

Finally, chapter 5 comprises some brief concluding remarks.