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New tools for the quantitative assessment of prodrug delivery and neurotoxicity

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ABSTRACT

Systemic off-target toxicities, including neurotoxicity, are prevalent side effects in cancer patients treated with a number of otherwise highly efficacious anticancer drugs. In the current study, we have: (1) developed a new analytical metric for the *in vivo* preclinical assessment of systemic toxicities/neurotoxicity of new drugs and delivery systems; and (2) evaluated, in mice, the *in vivo* efficacy and toxicity of a versatile and modular NanoDendron (ND) drug delivery and imaging platform that we recently developed. Our paclitaxel-carrying ND prodrug, ND^{PXL}, is activated following proteolytic cleavage by MMP9, resulting in localized cytotoxic chemotherapy. Using click chemistry, we combined ND^{PXL} with a traceable beacon, ND^{PB}, yielding ND^{PXL}–ND^{PB} that functions as a theranostic compound. *In vivo* fluorescence FRET imaging of this theranostic platform was used to confirm localized delivery to tumors and to assess the efficiency of drug delivery to tumors, achieving 25–30% activation in the tumors of an immunocompetent mouse model of breast cancer. In this model, ND-drug exhibited anti-tumor efficacy comparable to nab-paclitaxel, a clinical formulation. In addition, we combined neurobehavioral metrics of nociception and sensorimotor performance of individual mice to develop a novel composite toxicity score that reveals and quantifies peripheral neurotoxicity, a debilitating long-term systemic toxicity of paclitaxel therapy. Importantly, mice treated with nab-paclitaxel developed changes in behavioral metrics with significantly higher toxicity scores indicative of peripheral neuropathy, while mice treated with ND^{PXL} showed no significant changes in behavioral responses or toxicity score. Our ND formulation was designed to be readily adaptable to incorporate different drugs, imaging modalities and/or targeting motifs. This formulation has significant potential for preclinical and clinical tools across multiple disease states. The studies presented here report a novel toxicity score for assessing peripheral neuropathy and demonstrate that our targeted, theranostic NDs are safe and effective, providing localized tumor delivery of a chemotherapeutic and with reduced common neurotoxic side-effects.

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1. Introduction

The treatment of cancer patients with a variety of highly effective anti-neoplastic agents is generally accompanied by off-target systemic toxicities that limit the dosage, with neurotoxicity being one of the most severe and often dose-limiting non-hematological morbidities of chemotherapy (Cavaletti et al., 2011; Argyriou et al., 2012; Grisold et al., 2012). The neurotoxicity of anticancer drugs, including the widely used and efficacious platinum drugs and antitubulins such as paclitaxel (PXL), is most often manifested as peripheral neurotoxicity, commonly called peripheral neuropathy (Cavaletti et al., 2011; Grisold et al., 2012; Gradishar et al., 2005). Notably, patients treated with taxane-containing regimens including PXL or nab-PXL, an often prescribed nanoparticulate albumin-bound clinical formulation of PXL, frequently develop such peripheral neurotoxicity (Gradishar et al., 2005) that may present as the dose-limiting toxicity for these and other therapies. Neurological impairment, including sensory neuropathy, that results from treatment with a number of widely used anticancer drugs, is often such a severe and persistent side-effect that the dosage must be reduced or the drug replaced with alternate and potentially less-effective therapy (Grisold et al., 2012); thus, the potential benefit to patients of otherwise effective drugs can be abrogated by debilitating neurotoxicity.

There are methods to assess some drug-induced toxicities, for example, chemotherapy-induced liver toxicity can be revealed, both in preclinical studies and clinical practice, by measurements of plasma alanine transaminase (ALT) or aspartate transaminase (AST). By contrast, neurotoxicities are more difficult to detect and/or evaluate particularly during preclinical drug development. Unfortunately, despite the prevalency of drug-induced neurotoxicity that may result, at least in part, from off-target effects of anticancer drugs on peripheral nerves and the dorsal root ganglia of primary sensory neurons (Argyriou et al., 2012), the mechanisms that lead to this pathology remain poorly understood (Kudlowitz and Muggia, 2013; Gornstein and Schwarz, 2014). Thus, in practice, the off-target induction of peripheral neurotoxicity in either animals or patients on chemotherapy is not only challenging to predict, prevent and treat but also can be late-onset (Argyriou et al., 2008; Frigeni et al., 2011; Pachman et al., 2011; Grisold et al., 2012). This is a particular problem during preclinical testing of new drugs in small animals, since the methods to assess such chemotherapy-induced peripheral neurotoxicity in such models are limited. Consequently, the debilitating and persistent neurotoxic side-effects of new therapeutics and/or drug formulations often do not become evident until after initiation of clinical trials and sometimes not until after extensive clinical use (Grisold et al., 2012; Gradishar et al., 2005). Thus, not only is there is a critical need for drug formulations that reduce or eliminate peripheral neuropathy, but the problem is exacerbated by the lack of reliable preclinical tools to assess such chemotherapy-induced peripheral neurotoxicity or other off-target effects of new therapies. The work presented here addresses both of these issues.

Some of the systemic toxicities associated with cytotoxic chemotherapeutics can be ameliorated by formulation of drugs as nanomaterials (Davis et al., 2008; Ahmed et al., 2012; Kanapathipillai et al., 2012; Ranganathan et al., 2012) and/or as non-toxic and essentially inactive prodrugs that require *in vivo* activation to yield the cytotoxic agent, *e.g.*, *via* cleavage by proteinases, such as the matrix metalloproteinases (MMPs) (Zawilska et al., 2013). Using a design based on our proteolytic beacons (McIntyre et al., 2004, 2010; McIntyre and Matrisian, 2009), we recently demonstrated a PXL prodrug that is not toxic to tumor cells *in vitro* until activated by MMP9 (Samuelson et al., 2013). In these studies, we report the synthesis of a similar MMP9-activated PXL prodrug, ND^{PXL}, constructed on a clickable NanoDendron (ND) scaffold and an analogous proteolytic beacon, ND^{PB}. By linking ND^{PXL} to either ND^{PB} or another ND to enhance solubility, we have produced a set of prototypical MMP9-activated ND reagents (Fig. 1) that were tested for *in vivo* efficacy and toxicity in mouse models of breast cancer. Combined as ND^{PXL}–ND^{PB}, the MMP9-activated prodrug and imaging beacon NDs provide *in vivo* quantification of not only delivery of the ND to the tumor, but also an estimate of the activation of the prodrug at the tumor target from the measured cleavage of the intrinsic ND beacon. The self-reporting ND^{PXL}–ND^{PB}, a bifunctional theranostic (Kelkar and Reineke, 2011) agent, was used *in vivo* to measure the efficiency of tumor-associated activation of the prodrug in each of two preclinical models of

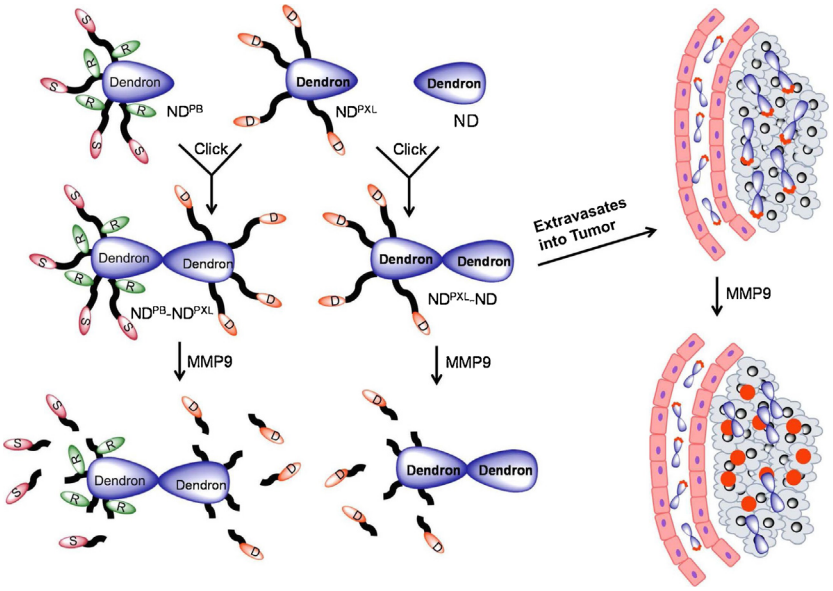

Fig. 1. Schematic representations of the NanoDendrons (NDs) structures and cleavage (left side) and mechanism of action (right side), illustrating NDs within the vasculature and extravasated into tumor tissue, an MMP9-rich microenvironment. The proteolytic beacon, ND^{PB}, includes both reference (R) and sensor (S) fluorophors; the prodrug, ND^{PXL}, carries PXL (D) attached to the MMP9-cleavable peptide (shown in heavy black).