Acoustic Cluster Therapy displays theranostic capability in enhancing the effectiveness of liposomal doxorubicin treatment of human triple negative breast cancer in mice

Nigel Bush
Joint Department of Physics
Institute of Cancer Research and Royal Marsden Hospital
London, UK
nigel.bush@icr.ac.uk

Andrew Healey
Phoenix Solutions AS
Oslo, Norway
ajhealey@online.no

Anant Shah
Joint Department of Physics
Institute of Cancer Research and Royal Marsden Hospital
London, UK
anant.shah@icr.ac.uk

Gary Box
Division of Cancer Therapeutics
Institute of Cancer Research
London, UK
gary.box@icr.ac.uk

Vladimir Kirkin
Division of Cancer Therapeutics
Institute of Cancer Research
London, UK
vladimir.kirkin@icr.ac.uk

Spiros Kotopoulis
Phoenix Solutions AS
Oslo, Norway
Spiros.kotopoulis@phoenixsolutions.no

Svein Kvåle
Phoenix Solutions AS
Oslo, Norway
svein.kvale@phoenixsolutions.no

Annemieke van Wamel
Department of Physics
NTNU
Trondheim, Norway
annemieke.wamel@ntnu.no

Catharina de Lange Davies
Department of Physics
NTNU
Trondheim, Norway
catharina.davies@ntnu.no

Per Christian Sontum
Phoenix Solutions AS
Oslo, Norway
Per.Sontum@phoenixsolutions.no

Jeffrey Bamber
Joint Department of Physics
Institute of Cancer Research and Royal Marsden Hospital
London, UK
jeff@icr.ac.uk

***Abstract*—Acoustic Cluster Therapy (ACT) employs an intravenously-injected dispersion of clusters of microbubbles and oil microdroplets, in combination with ultrasound directed at the tumor, to enhance the delivery of a co-administered drug. Diagnostic ultrasound activates the clusters, causing the droplets to change phase and become large (> 20 μm) bubbles which lodge in the tumor capillaries. Delivery-enhancement is then achieved using a low-frequency (e.g. 300 kHz) ultrasound field also directed at the tumor, which causes the stationary bubbles to pulsate whilst in direct contact with the vascular endothelium. ACT has been shown to significantly increase the efficacy of various chemotherapeutics in a variety of tumor models in mice. Here we demonstrate through preclinical *in-vivo* experiments that the efficacy of treating human triple negative breast cancer in mice with liposomal doxorubicin (Doxil®) is significantly enhanced by ACT; for example, 63 % of animals achieved complete, stable remission, in the group treated with the drug and ACT versus only 10 % for Doxil® alone ($p < 0.02$). We also show in the same experiments that the ACT contrast enhancement obtained under ultrasound activation of the ACT clusters, either before or during treatment, holds promise as an imaging biomarker for predicting response.**

Keywords—ACT, cancer treatment response, drug delivery, imaging biomarker, microbubble

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I. INTRODUCTION

Acoustic Cluster Therapy (ACT) is designed to overcome some of the limitations of conventional microbubbles for enhancing drug delivery in combination with ultrasound [1, 2]. It involves co-administration of a dispersion of clusters containing a microbubble and an oil microdroplet [3], together with an unmodified medicinal drug such as a chemotherapeutic agent. Diagnostic ultrasound directed at the tumor to be treated vaporizes the droplets, turning the clusters into bubbles of diameter > 20 μm. These large bubbles lodge in, and possibly occlude, tumor capillaries for many minutes, during which time low-frequency ultrasound (e.g. 300 kHz) is applied to make them pulsate, enhancing the delivery of any co-administered drug via a range of biomechanical effects. This has been shown to significantly increase therapeutic efficacy of a number of anticancer drugs in various tumor models in mice [4-7].

In this study we (a) investigated the efficacy of liposomal doxorubicin (Doxil®) with and without ACT for treatment of human, triple negative, breast cancer in mice, and (b) evaluated whether the ACT-activation signature reported in a companion paper [2] has value for predicting individual therapeutic outcome.

II. Materials and Methods

A. Animal groups

Clinical grade liposomal doxorubicin, Doxil® (6 mg/kg, maximum tolerated dose) referred to here as DOX, was given intravenously to athymic mice when tumors (MDA-MB-231-luc orthotopic human triple negative breast cancer xenografts) had reached a volume of about 209 mm^3, and again 21 days later, either alone or immediately followed by three sequential intravenous injections of the ACT cluster-dispersion known as PS101. PS101 consisted of a commercially available microbubble (Sonazoid™, GE Healthcare) and a microdroplet of perfluoromethylcylopentane (PFMCP, F2 Chemicals Ltd, UK). Animals were randomized into the following three groups, depending on when tumors reached the starting volume (7 to 10 per group).

- Group 1 (PS101+US): 9 animals, no drug, 3 doses of PS101 of 2 mL/kg at the first treatment and 3 doses of PS101 of 2 mL/kg 21 days later, exposed to ultrasound.
- Group 2 (DOX): 10 animals, Doxil® 8 mg/kg at first treatment and 6 mg/kg at second treatment, no PS101, not exposed to ultrasound.
- Group 3 (ACT+DOX): 7 animals, Doxil® 6 mg/kg at first treatment and 6 mg/kg at second treatment, 3 doses of PS101 2 mL/kg at the first treatment and 3 doses of PS101 2 mL/kg 21 days later, exposed to ultrasound.

B. Treatment

The apparatus for handling the mice, ultrasound imaging and delivery-enhancement insonation is described in a companion paper in these proceedings [2]. In this study, however, mice were placed in a dorsal recumbent position. For each PS101 injection, microdroplet vaporization was achieved by 45-s B-mode scanning of the tumor at 8 MHz and a mechanical index (MI) of 0.33 [8], using a Toshiba Aplio XG scanner and 1204BT transducer. Low-frequency insonation then occurred for 5 min at 300 kHz and an MI of 0.15 using a custom made, single element bowl transducer (Imasonic) focused towards the tumor.

C. Data analysis

The ultrasound fundamental echo B-mode tumor ACT-activation contrast enhancement [2] was determined from the ultrasound images recorded during activation and was given a visual ranking. This was repeated for all three PS101 injections on the first treatment, and all three injections 21 days later. The average score (higher value indicates increased contrast and activation) was compared with therapeutic response in a blinded manner. To score therapeutic response, non-responding animals (sacrificed prior to 175 days) were ranked by survival time (shorter survival = lower rank) and responders were ranked by the day that the tumor volume reached (and stayed at) zero (shorter time to reach zero = higher rank).

III. Results and Discussion

ACT substantially improved the therapeutic efficacy of Doxil®, resulting in 63 % of animals in complete, stable remission, versus 10 % for Doxil® alone ($p < 0.02$). As expected, no therapeutic response was observed in the PS101+US group, where median survival was 21 days. This improved significantly to 67 days for the DOX group ($p = 0.04$ for comparison with the PS101+US group), and again improved with many animals still surviving at the end of the study in the ACT+DOX group ($p = 0.0004$ for comparison with the PS101+US group). In the ACT+DOX group, a heterogeneous population of responses was observed, facilitating assessment of the ACT-activation contrast score against tumor response.

The B-mode ACT-activation contrast score positively and strongly correlated with response rank ($r = 0.96$, $p = 0.0005$) (Fig. 1), indicating that ACT has theranostic potential such that its use during or before treatment may be employed as a predictor of therapeutic response, potentially for patient management during future clinical use.

IV. Conclusion

The use of ACT with Doxil® substantially improves therapeutic response of human triple negative breast cancer in mice over treatment with Doxil® alone. This was observed by

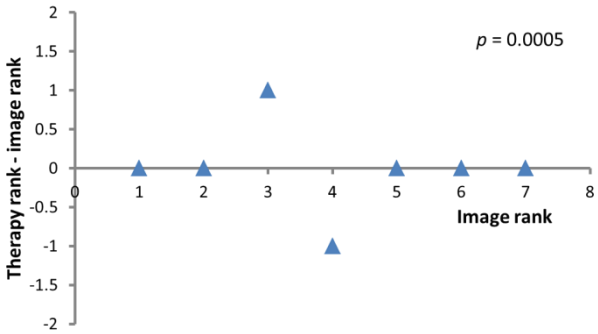

Figure 1. Therapy rank minus image rank plotted against image rank for all tumours in the ACT+DOX group. Perfect prediction of the level of therapeutic response by the image rank would always yield zero for the therapy rank minus image rank value. The probability that there was no correlation between image rank and therapy rank was 0.0005, assessed using a nonparametric Pearson's correlation, r = 0.96.

measurements of both tumor size and overall survival.

The ACT-activation contrast score appears to represent a useful biomarker for the prediction of therapeutic response which may have value for patient stratification and management. Future work to numerically quantify and automate the measurement of such indices would assist clinical translation of this potentially promising approach to improving treatment outcome.

CONFLICT OF INTEREST STATEMENT

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