

**Radiotherapy sensitization with ultrasound-stimulated intravenously injected oxygen  
microbubbles can have contrary effects depending on the study model**

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26 ***To the Editor:***

27         We are writing to share our collective experience over the last few years working on  
28 radiosensitization strategies using intravenously-administered, ultrasound-stimulated, oxygen  
29 microbubbles. There has been considerable preclinical success reported to date, including from  
30 our own groups, using oxygen microbubbles as a cancer theranostic agent to improve  
31 radiotherapy, sonodynamic therapy and brachytherapy treatments (1-4). However, we have  
32 recently come across several unexpected findings that warrant further study and caution as these  
33 techniques move towards translation. As we work toward publishing these in the coming months,  
34 we believe that their implications are important to communicate quickly and therefore outline  
35 key observations in this letter.

36         In particular, we have observed that oxygen microbubbles can increase, or conversely  
37 decrease, radiotherapy therapeutic efficacy depending on the preclinical tumor model used. We  
38 have established that these findings are reproducible and unrelated to microbubble shell  
39 formulation and anesthesia type (inhaled isoflurane carrier gas or injectable anesthesia), but may  
40 be ultrasound-mediated and tumor model dependent. Specifically, we have observed that  
41 intravenously administered lipid-shelled oxygen microbubbles, formulated as in (5), in  
42 combination with ultrasound stimulation improved tumor control after radiation therapy in an  
43 immunodeficient mouse model of CAL27 head and neck squamous cell carcinoma (model  
44 described in (6, 7)). However, in contrast, when the same treatment was applied in an  
45 immunocompetent, fibrosarcoma tumor allograft Fisher 344 rat model —a model in which we  
46 had previously observed improved tumor control after radiation therapy with direct tumoral

47 injection of lipid-shelled oxygen microbubbles (2)—, the technique demonstrated significantly  
48 worsened tumor control after radiotherapy compared to radiotherapy alone. Interestingly,  
49 administration of oxygen microbubbles in the absence of ultrasound sonication did not result in a  
50 difference in tumor control after radiotherapy in this fibrosarcoma model. Similarly, a second  
51 microbubble formulation, surfactant-shelled oxygen microbubbles as in (8), have also  
52 demonstrated increased radiotherapy efficacy in immunodeficient mice, in both subcutaneous  
53 and orthotopic breast cancer models, as well as the CAL27 squamous cell carcinoma model (1, 7,  
54 9). However, a similar negative influence on radiosensitivity was observed again in the rat  
55 fibrosarcoma model. A variable response has also been observed in pancreatic tumor models. In  
56 investigations of radiotherapy in combination with sonodynamic therapy using lipid-shelled  
57 oxygen microbubbles, a statistically significant improvement in tumor response was seen in  
58 PSN-1 tumors, but not BxPC3 (10). This was initially surprising because with sonodynamic  
59 therapy alone, oxygen microbubbles were found to produce an enhanced response in BxPC3  
60 tumors (3). One possible explanation, however, is the better vascularization of BxPC3 tumors  
61 leading to a less pronounced synergistic effect of sonodynamic therapy with radiotherapy due to  
62 lower radioresistance.

63 In light of these findings, which show that radiotherapeutic response to oxygen  
64 microbubbles is model dependent, our laboratories wish to raise awareness of these potential  
65 unexpected negative consequences in select cases. This variability in efficacy is also apparent  
66 across tumor types in clinical trials using microbubbles to augment chemotherapy (11-13), and  
67 highlights the importance of selecting appropriate preclinical models prior to clinical translation  
68 (14). We hereby suggest that researchers in the field examine these effects and models in more  
69 detail, focusing on the underlying tumor physiology, as well as the interactions between the

70 ultrasound, microbubble and biological processes, to elucidate the underlying mechanisms  
71 involved.

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## 78 Conflict of Interest Statement

79 Virginie Papadopoulou, Mark Borden, Paul Dayton, and John Eisenbrey are all inventors or co-  
80 inventors on patents related formulations and applications of oxygen microbubbles and other  
81 types of contrast agents. Paul Dayton is a co-founder of Triangle Biotechnology, Inc., and a co-  
82 inventor on patents licensed by Triangle Biotechnology. Paul Dayton is also a scientific advisor  
83 for SonoVascular. Mark Borden is a Founder and the Chief Scientific Officer of Respirogen Inc.  
84 (Boulder, CO). Eleanor Stride is a founder and scientific advisor to SonoTarg Ltd. The other  
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## 88 Data Availability Statement

89 Not applicable.

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