Abstract and Introduction

Remove IMRT from the abstract, wrote full form, readers cannot understand in short form when it come such early in abstract.

How author envision to in vitro in vivo extrapolation of the method developed for the real time cancer radiotherapy? What readout author can think about to help the track radiotherapy in real cancer radiotherapeutic regimes? In this context, putting a concluding statement in the abstract will add value to this work.

In **Introduction,** Cite a latest report https://doi.org/10.1021/acs.chemrestox.1c00219 to refer the sentence ending with 'large tissue culture vessels and laboratory irradiators are widely used for this purpose', which emphasizes how advances in in vitro methods could potentiate prediction model for in vivo therapeutic and toxicological effects.

While enumerating various advantages, I suggest authors also put some important for such in vitro validated HTP platform development is useful because 1) in vitro HTP models allow the use of cells from human sources, avoiding possible inter-species differences related to animal models; 2) Such in vitro radiotherapy model rae useful since the Ionizing radiation (IR) damage to endothelial cells or ECs composing the microvasculature is particularly troublesome since ECs are present both in tumor and healthy tissues, playing an essential role in shaping the microenvironment; 3) Such in vitro platforms are key to obtaining information on potential molecular targets for the setting up of novel radiosensitization approaches or for the design of mathematical algorithms suitable for tailoring radiotherapy as shown lately https://doi.org/10.1007/s11684-020-0761-1.

Materials and Methods

Provide catalogue number and source of all materials and model/version supplier of all instruments used in the study.

For the accurate in vitro analysis using different imaging and Chemianalytics platform in tissue culture plate (6, 24, 96 well), the design accuracy is paramount important for calibration and standardizing the experiment. Therefore, the Plate irradiation setup development needs in depth info. I encourage authors to provide CAD model with full details in materials method, as authors give a

cartoon in figure 1.A is not sufficient. Provide dimension details and design parameters for 3 types of culture plate tested in this report.

Are clinical linear accelerator automated as shown in fig.1 C-D or authors have to adjust the platform manually?

Fig. 1 caption "Plates positioned with aid of multiwall tissue culture plate set-up and room lasers.", please rephrase as it is unclear.

Results and discussion

Are wells filled with any liquid as shown In Figure 2. A, B & C. Coronal view of 96, 24 and 6 well tissue culture plates showing contouring of wells? Do authors think the flattening meniscus of well from 96 to 6 well can make any imaging artifacts which could be in decisive for the analysis?

Statement in discussion section 'In order to screen the large number of potential radiation-drug combinations quickly and efficiently, there is a need for a high-throughput, in-vitro radiobiology platform' needs a suitable reference, cite a Latest report doi:10.15761/FNN.1000S2006 regarding HTP platforms changing the landscape of clinical research in this context.

In Table 1. Radiation dose statistics of multiwall tissue culture plate IMRT plans, how the '0' Gy target dose wells have some numbers in mean/max/min calculated, please clarify it is confusing.

Give details how 'predicted' doses were calculated, did author used and mathematical prediction tools, provide details please?

Provide high-resolution plots with bigger font size to make data readable. Data visualization is really poor quality.

Add scale bars to all images.