Comparing equals when evaluating immunotherapy with different doses and fractions of radiation therapy

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The combination of immunotherapy with radiation therapy is showing evidence of therapeutic synergy. Exciting preclinical data and the emergence of new clinically available immunotherapies has led to an explosion of combination therapies in the clinic. To understand the synergy of radiation and immunotherapy, increasing effort is going into investigating mechanisms of interaction in preclinical models. However, in one aspect we do not come close to modeling standard clinical scenarios. Few studies in mice recapitulate standard fractionation as given to patients. We discuss the problem in modeling clinically relevant biological equivalent dose in preclinical studies of immunotherapy and radiation therapy.

As is well known in radiation oncology, repeated doses of radiation do not necessarily scale in a linear manner, such that 2 Gy delivered in five daily doses is not equivalent to 10 Gy in a single dose. In order to compare efficacy of different radiation regimens, an equation was developed to explain the biologic equivalent dose, BED, based on the in vivo survival curve. The BED equation is:

\[ \text{BED} = n(d + \frac{d\alpha}{\beta}) \]

where \( n \) is the number of fractions, \( d \) is the dose per fraction, \( \alpha \) is the intrinsic radiosensitivity of the cells corresponding to the lethal events from a single particle and where \( \beta \) is the sublethal damage caused by two charged particles. The \( \alpha/\beta \) ratio characterizes the cell type, for instance the spinal cord is assumed to have an \( \alpha/\beta \) of 2–3, which means this tissue is slowly proliferating and late reacting. Conversely, intestinal epithelial cells have an \( \alpha/\beta \) of 10 corresponding to rapid proliferation and early response. To provide an example of equivalent BED, we have demonstrated synergy between radiation therapy and immunotherapy in mice using radiation doses of 20 Gy in a single fraction [1], which is a total dose of 20 Gy but a BED\(_{10}\) of 60. This dose has also shown dramatic results in treatment of patients with metastatic melanoma in combination with immunotherapy with systemic IL-2 [2]. To compare with conventional fractionation in 1.8 Gy doses, we would need to provide 28 treatments, which is a total dose of 50.8 Gy and a BED\(_{10}\) of 59.5 Gy. This represents over 5 weeks of daily weekday radiation therapy to tumor-bearing mice to achieve an equivalent BED, which can be problematic in scheduling and exceeds the standard time-course of many mouse experiments. There are studies that match the total dose split across fractions, but there are few studies using low-fraction sizes in combination with immunotherapy that approach the BED achieved with large fraction sizes. Most preclinical studies use single doses or short courses of radiation therapy across 1–5 fractions. This is more equivalent to stereotactic body radiotherapy (SBRT),

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Michael J Gough
Author for correspondence:
Earle A Chiles Research Institute,
Robert W Franz Cancer Center,
Providence Portland Medical Center, 4805 NE Glisan St, Portland, OR 97213, USA
Tel.: +1 503 215 3928
michael.gough@providence.org

Marka R Crittenden
Earle A Chiles Research Institute,
Robert W Franz Cancer Center,
Providence Portland Medical Center, 4805 NE Glisan St, Portland, OR 97213, USA
and
The Oregon Clinic, Portland, OR 97213, USA

Kristina H Young
Earle A Chiles Research Institute,
Robert W Franz Cancer Center,
Providence Portland Medical Center, 4805 NE Glisan St, Portland, OR 97213, USA
and
The Oregon Clinic, Portland, OR 97213, USA
a term for treatment of extracranial tumors with five or fewer treatments of radiation where daily doses can range from 6 to 20 Gy.

It should be noted that the BED equation may not be accurate for the larger fraction sizes (>5 Gy) used in SBRT [3]. The basis of the linear-quadratic (LQ) equation, on which the BED is calculated, is that all clonogenic cell death occurs as a result of DNA damage. However, one outcome of increased fraction sizes is that normal cells in addition to cancer cells can be killed as doses increase. Death of certain key populations of noncancer cells can have knock-on effects. For example, death of endothelial cells can destroy the blood supply to the tumor, and these cells are sensitive only to larger radiation fraction sizes. Purified endothelial cells have been shown to undergo apoptosis only beyond a threshold of 11 Gy in a single fraction, and apoptosis then increased with dose [4]. Hellevik and Martinez-Zubiaurre recently reviewed the responsiveness of a range of stromal populations to radiation therapy [5] and also explore the literature showing how tumor-associated fibroblasts, while extremely resistant to radiation-induced death, are highly responsive to radiation in both phenotype and secretory patterns. Together, these stromal responses may be dose dependent and unrelated to DNA damage-related death of cancer cells. Several modifications to the LQ equation have been suggested to better model cell death at higher doses per fraction including the universal survival curve (USC), which adds higher additional terms beyond the d2, or to assume a larger α/β ratio for tumor cells.

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In patients, traditional fractionated radiation is classically thought to be immunosuppressive at the treatment site in part because lymphocytes are sensitive to even low-radiation doses and are cleared with each treatment. Since lymphocytes are killed at almost any therapeutic dose that kills cancer cells, further increasing the dose of radiation is likely to be of little influence to their clearance, but extending the course of treatment from days to weeks may have significant consequence to emerging immune responses in the tumor. However, radiation therapy also results in rapid attraction of T cells back to the treatment site. This attraction of T cells has been exploited to direct adoptively transferred tumor-specific T cells to the irradiated tumor, such that tumor control was dependent on 10 Gy × 1 (BED_10 of 20 Gy) radiation therapy in association with adoptive T-cell transfer [6]. These authors further demonstrated that radiation therapy results in dose dependent induction of major histocompatibility complex class I (MHCI) on cancer cells in vitro, with no effect at 1 or 4 Gy, but induction at 10 and 25 Gy [6]. Each increase in dose also increased the time that the transporter associated with antigen processing (TAP) was saturated with peptide, and single doses of 8 Gy or 10 Gy but not 2 Gy sustained elevated MHCI in cells for over 10 days [6]. In addition, radiation can affect the phenotype of tumors rendering them more susceptible to T-cell killing. In vivo radiation of tumors with 8 Gy × 1 (BED_10 of 14 Gy) was shown to upregulate Fas on the surface of cancer cells [7]. In this model, radiation following tumor-associated antigen vaccination significantly increased T-cell infiltration to tumors and only the combination resulted in tumor cures at either a dose of 8 Gy × 1 (BED_10 of 14 Gy) or 2 Gy × 4 (BED_10 of 9.6 Gy) [7]. On the other end of the dose range, Klug et al. [8] demonstrated in a murine spontaneous insulinoma model that even doses of radiation as low as 0.5 Gy were able to attract T cells to malignant lesions in the pancreas. In combination with adoptive transfer of tumor antigen-specific T cells, 2 Gy × 1 (BED_10 of 2.4 Gy) of radiation to the pancreas was able to permit survival of all animals, while animals in other groups did not survive [8]. Thus, radiation-mediated attraction of pre-existing T cells to tumor lesions may not require dramatic cancer cell death or modulation of their phenotype.

Many of the current research efforts in immunotherapy with radiation therapy are testing antibodies that block or activate checkpoint molecules on T cells in combination with radiation, and similarly here a wide range of doses have been tested (reviewed in [9]). For example, Dewan et al. demonstrated that in the TSA mammary cancer model and MCA38 colorectal cancer model, fractionation of radiation into 8 Gy × 3 (BED_10 of 43 Gy) was most effective in both local control and control of distant tumors when combined with anti-CTLA4 immunotherapy [10], but that schemes that gave higher BED – 6 Gy × 5 (BED_10 of 48 Gy) or 20 Gy × 1 (BED_10 of 60 Gy) – were significantly less effective by the day 35 end point of the experiment [10]. This optimum may need to be empirically determined with different tumor models, as in the 4T1 mammary carcinoma model, both 12 Gy × 1 (BED_10 of 26 Gy) and 12 Gy × 2 (BED_10 of 52Gy) demonstrated synergy with anti-CTLA4 immunotherapy [11], though in this model the combination does not extend survival [12]. Ghandi et al. recently reviewed the literature to examine the effect of dose and fractionation in combination with immunotherapy [9], and we agree with their conclusion that the currently published preclinical
studies “do not give a consistent answer as to the best fractionation scheme to use.”

An alternative to modeling conventional fractionation in mouse models of cancer, is to use the classic TCD50 analysis (dose to produce 50% tumor cures) to compare responses to a controlled range of doses. For example, Milas et al. nicely demonstrated synergy between intratumoral CpG administration and radiation at 20 Gy × 1 (BED10 of 60 Gy) in immunocompetent mice [13]. Importantly, the authors also constructed TCD50 curves from 10 to 55 Gy in single doses in the presence and absence of intratumoral CpG, demonstrating a shift in TCD50 from approximately 40 Gy × 1 (BED10 of 200 Gy) with radiation alone to approximately 20 Gy × 1 (BED10 of 60 Gy) when combined with CpG [13]. The same group performed a similar series of experiments using fractionated radiation, where mice were given ten total fractions, but each fraction varied in size from 1 Gy (BED10 of 11 Gy) to 9 Gy (BED10 of 171 Gy) [14]. These experiments demonstrated a clear shift in the total dose required for TCD50, from 8 to 9 Gy × 10 (BED10 144–154 Gy) for radiation alone to 2–3 Gy × 10 (BED10 24–39 Gy) when combined with CpG [14]. Such approaches provide a direct assessment of the contribution of immunotherapy to tumor control by radiation therapy, and also inform whether there is a cut-off of total dose or fraction size for synergy.

In clinical studies, immunotherapy is being tested in combination with both conventionally fractionated and hypofractionated regimens. We currently do not know enough to assume that immunotherapy will synergize with any fractionation regimen, but the majority of new studies use SBRT fractionation schemes that have been modeled in mice. In view of the expanding array of immunotherapy approaches reaching patients, it is reasonable to assume that as their mechanisms of action vary their synergy with radiation will also vary. While perhaps immunological adjuvants such as TLR ligands may synergize well with large-scale antigen release through high doses of radiation, immunotherapies targeting T cells may only need smaller doses to direct T cells to treatment sites. While this is speculation, preclinical comparisons using consistent BED will improve our ability to optimize the immune consequences of radiation therapy.

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