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EDITORIAL

Radiotherapy, tumor mutational burden, and immune checkpoint inhibitors: time to do the math

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Immune checkpoint inhibitors (ICI) are revolutionizing lung and skin cancer therapy [1–7], and there is accumulating evidence for a further significant and highly relevant boost of progression-free and overall survival once these substances are combined with radiotherapy (RT) [8, 9].

The tumor mutational burden (TMB) and the corresponding increase in neoantigen formation subsequent to exon mutations correlate with response after treatment with ICI [10, 11]. Large studies of mutational patterns have shown the highest TMB in melanoma, lung, and bladder cancers, although considerable variation is present within individual tumor entities [12, 13]. In this regard, the CheckMate 227 trial recently established a TMB of ≥10 mutations per 10^6 bases (=1 megabase; Mb) as a robust and independent biomarker of response [14]. These data recently led radiation oncologists to speculate whether the improved efficacy of ICI plus RT may be caused by radiation-induced TMB.

We propose to take a closer look at this hypothesis by performing a simple calculation: Assume that 40 DNA double-strand breaks (DSBs), 1000 DNA single-strand breaks (SSBs), and 3000 base lesions are induced per Gy in the genome [15]. SSBs and base lesions arise in their thousands every day as a result of physiological processes in all cells and are repaired with extremely high efficiency. Even the vast majority of DSBs are repaired so that less than one DSB remains (per cell) after a daily fraction size of 2 Gy. However, induced mutations adding to the TMB result from incorrectly repaired rather than unrepaired lesions. Approximately 15% of DSBs are repaired by error-free homologous recombination (HR) and the rest by non-homologous end joining (NHEJ) which may introduce small deletions or insertions ("indels") if simple end joining is not possible. This may result if end resection occurs or spurious homologies are present. Furthermore, misrepair may join unrelated DSB ends and fuse different genes or chromosomes. Assuming a probability of 10% for indels and a ratio of 8:1 base substitutions [16], a typical RT scheme of 60 Gy in 30 fractions of 2 Gy might produce $8 \times 30 = 240$ indels and approximately 2000 base substitutions. In the worst case, where most DSBs repaired by NHEJ would lead to indels, $50 \times 30 = 1500$ indels and thus 12,000 base substitutions might be produced. The range of 240–1500 indels is consistent with the range of 135–943 indels and 6–321 rearrangement break points found per genome in 12 radiation-induced tumors [16]. Considering that the genome contains 6.6×10^9 bases, a standard RT scheme is unlikely to introduce more than 1 mutation per Mb.

Thus, in standard-fractionated RT, it is unlikely that radiation-induced DNA modifications sufficiently raise the MTB above the critical limit of 10 per Mb.

In the case of RT using very large doses per fraction (such as in Stereotactic Body Radiotherapy [SBRT], Stereotactic Radiosurgery [SRS], brachytherapy or Intraoperative Radiotherapy [IORT]), error-free DNA damage repair becomes increasingly saturated and the rate of misrepaired DNA alterations may be considerably higher [17]. In line with this, clinical evidence arises from studies on ICI combined with SRS or SBRT that showed impressive responses and improved overall survival rates [18–23]. However, until quantitative yields of misrepair after high single doses become available, it is not possible to assess whether the MTB is high enough to explain these results. An alternative interpretation of the data is the function of RT as an immunological adjuvant creating an anti-tumor vaccine based on existing tumor antigens via necrotic or immunogenic cell death associated with an inflammatory response [24–29].

RT increases TMB, but the math does not support the hypothesis that conventionally fractionated RT increases the MTB enough to induce exploitable tumor antigens. The increase in TMB after a course of conventionally fractionated RT is one magnitude below the proposed threshold of 10 mutations per Mb for the induction of immune ef-

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fects in combination with ICI, indicating that other factors may be relevant for RT-induced immune stimulation [30]. It is plausible that the picture is different after high single doses of RT, where alternative mechanisms may explain the interaction of RT and ICI.

Conflict of interest F.A. Giordano, M.R. Veldwijk, C. Herskind, and F. Wenz declare that they have no competing interests.

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