systemic anti-PD1 was able to significantly improve abscopal effect in 344SQR murine metastatic lung cancer model, most of the mice eventually died due to the growth of secondary tumors. Therefore, we intended to use HD-XRT plus NBTXR3 injection into primary tumors and low-dose (LD) radiation on secondary tumors plus dual-agent immunotherapy (IT) of anti-PD1 and anti-CTLA-4 to achieve complete control of both the primary and secondary tumors in mice.

Methods Five groups of 8 mice each were inoculated subcutaneously with 5 × 104 anti-PD1-resistant 344SQR cells in each hind leg, 3 days apart, to establish ‘primary’ (right) and ‘secondary’ (left) tumors. All mice in treatment groups received intraperitoneal anti-PD1 and anti-CTLA-4 on days 4, 7, 10, and 13, and continuing anti-PD1 treatment on days 20, 27, 34, 41, and 49 and 12 Gy x3 (HD-XRT) to the primary tumors on days 7, 8 and 9. Primary tumors in groups 3 and 5 also received intratumoral NBTXR3 on day 6. Secondary tumors in groups 4 and 5 were also irradiated with 1 Gy x2 (LD-XRT) on days 12 and 13. Experimental groups were designated as 1 = Control, 2 = HD + IT, 3 = NBTXR3 + HD + IT, 4 = HD + LD+IT, and 5 = NBTXR3 + HD + LD + IT. The secondary tumors were analyzed by flow cytometry and Nanostring. On day 178, the survivor mice were rechallenged with 5 × 104 344SQR cells on the right flank and the tumor growth was monitored for an additional 36 days.

Results All mice in all the groups except NBTXR3 + HD + LD + IT died due to the growth of either the primary tumor or the secondary tumor by day 36. Both the primary and the secondary tumors in 4 mice of NBTXR3 + HD + LD + IT group were completely eliminated. No tumor growth was observed in these mice after rechallenged with 344SQR cells. Flow cytometry data demonstrated that only the mice in the groups with NBTXR3 had significantly more CD8+ T cell infiltration in the secondary tumor collected on day 16 than the control. Both flow cytometry and Nanostring data showed that only the mice in NBTXR3 + HD + LD + IT had a significantly higher CD8+ Tcell/Treg cell ratio than the control.

Conclusions The combination of NBTXR3 plus high and low dose radiation with immunotherapy effectively controlled the growth of both primary and secondary tumors, significantly extending the survival, generating long-term antitumor memory. This combination therapy induced immune-mediated control of the secondary tumor at both genetic and cellular levels.

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