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 × 10^4 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 1Gy 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 × 10^4 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+ Tcell 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 extended the survival, generating long-term tumor immunity. This combination therapy induced immune-mediated control of the secondary tumor at both genetic and cellular levels.

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**References**


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Methods BxPC-3, PANC-1, and MiaPaCa2 were incubated alone or in combination with Tinzaparin (T) and/or Nab-Paclitaxel (A) and/or Gemcitabine (G) and/or Nivolumab (NI), Pembrolizumab (PE) and/or Ipilimumab (IPI). The effect of these regimes on various signaling pathways controlling proliferation and apoptosis was identified in vitro through Western blot. Cell viability was measured with MTT assay. NOD/SCID mice will be used to generate xenografts with the PANC-1 cell line. Human peripheral blood mononuclear cells (PBMCs) from healthy donors will be injected to give mice a human-like immune system.

Results In a triple combinational scheme, N/PE+I+P, the protein levels of VEGFR2 were decreased (0.1 to 0.7 folds) in a dose-dependent way in mtkRAS PC cell lines (PANC1 and MIAPACA2). The number of PANC-1 cells was decreased around 40% in a triple combinational scheme of T+I+P+(NI or PE) after 48 hours. The triple combination of Gemcitabine + Nab-paclitaxel + Tinzaparin leads to a decrease in tumor size relative to control by 51% and relative to Nab-P + G by 15%. The combination of chemotherapy, immunotherapy, and Tinzaparin leads to a reduction in tumor size compared to control by up to 60%. Tinzaparin contributes an additional 20% Preliminary data show that the quadruple therapeutic regimen increases the percentage of CD8+ cells from 5% to 27% and decreases Tregs’ percentage from 9.5% to 4% (in TILs).

Conclusions In vitro experiments show a decrease in the cell viability of PC cell lines and a reduction in the protein levels of VEGFR2 in mtkRAS PC cell lines. In vivo experiments with NOD/SCID mice and humanized NOD/SCID mice show a significant reduction in tumor volume in the combination therapy regimes with Tinzaparin. Possible mechanisms for these effects include an increase in CD8+ cells, a decrease in Tregs cells, a reduction in VEGFR-2 expression, and an increase in cancer cell apoptosis. This synergistic strategy can create new avenues for the treatment of patients with pancreatic cancer, achieving a better clinical outcome and greater survival.

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