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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Immune Checkpoint Blockade Promotes Thrombosis Via T-Cell and Neutrophil Activation, and Tumor-Cell Associated Tissue Factor (TF) in a Murine Model of Colorectal Cancer

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Background:
Immune checkpoint blockade (ICB) is a paradigm shift in cancer therapy. Despite clinical benefit, various adverse events are associated with ICB using immune checkpoint inhibitors (ICI). Venous thrombosis is an emerging clinical problem in cancer patients receiving ICI. Little is known about how ICI promotes thrombosis in cancer. To define the underlying mechanisms of ICI-associated thrombosis (IAT), we assessed the ICI impact on blood hypercoagulability and tumor TF expression using a mouse thrombosis model of colorectal cancer.

Methods:
We developed a CT26 mouse thrombosis model with ICI (aPD-1+aCTLA4) administration. Systemic hypercoagulability was assessed by measurement of circulating nucleosomes (Nu.Q H3.1 Immunoassay), neutrophil extracellular traps (NETs) (CitH3 immunofluorescence), circulating neutrophil-platelet aggregates (immunophenotyping), and levels of thrombin-antithrombin (TAT) complexes in ICI-treated mice. Tumor-associated TF and TF activity in circulating extracellular vesicles (EV) were assessed by western blotting and Factor Xa generation assays. To gain mechanistic insights into TF induction by ICI, tumor cytokines were also profiled (proteome profiler array). The role of cancer cell-derived TF in IAT was determined using mice implanted with CRISPR/Cas9-mediated TF knockout (TFKO) CT26 tumor cells.

Results:
ICI treated tumor-bearing mice developed larger thrombi than control mice and demonstrated elevated levels of circulating nucleosomes (114 *vs* 82 ng/ml), NET (15.8 *vs* 6.8%), platelet-neutrophil aggregates (44.8 *vs* 22.3%), and TAT complexes (12.6 vs 7.9 ng/ml) compared to IgG-treated mice. Tumor TF expression was increased in tumor extracts. Cytokine profiling suggested enhanced production of IFNɣ (2-fold) and TNFα (5-fold), as well as CXCL11 (6.8-fold), an effector T cell-recruiting chemokine. IFNɣ-increased TF expression in cultured CT26 cells, suggesting that T-cell derived IFNɣ may play a role in enhancing tumor TF expression in vivo. Circulating EV TF antigen and procoagulant activity were increased by ICI. CRISPR/Cas9-mediated TF KO in CT26 cells resulted in complete loss of TF expression, and mice bearing these cells showed reduced tumor and EV-associated TF (Fig. A), associated with a significant decrease in thrombus formation after ICI treatment (24.2 vs 20.5 mg, Fig. B). There was no significant difference in growth of wild-type and TF KO CT26 tumors.

Conclusions:
Our findings provide insights into cellular and molecular mechanisms associated with ICI-induced thrombosis, and suggest a role for neutrophil and granulocyte activation, and tumor-associated TF. Additional studies are ongoing to further define these pathways in the murine model and in patients.

Disclosures Khorana: *Pfizer:* Honoraria; *Sanofi:* Honoraria; *Anthos:* Honoraria; *WebMD:* Honoraria; *BMS:* Honoraria; *Bayer:* Honoraria; *Janssen:* Honoraria.

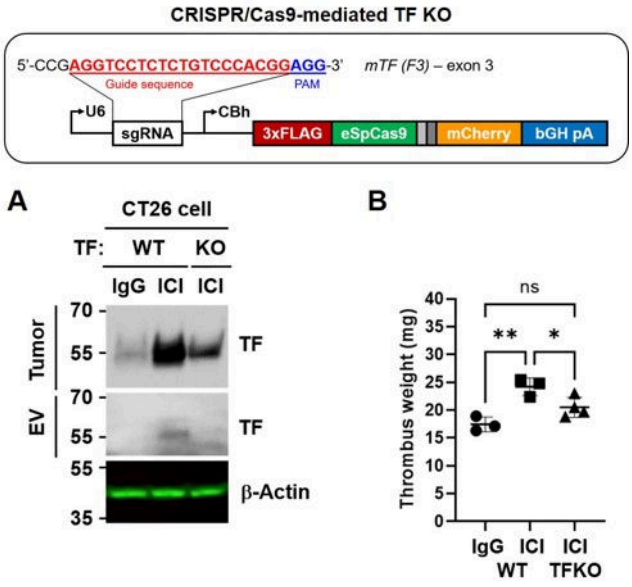

Figure 1

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