Butyrophillin-3A is expressed in multiple solid tumors: translational research supporting the EVICTION study with ICT01, an anti-BTN3A mAb activating V9gVd2 T-cells

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Background Butyrophillin-3A (BTN3A) three isoforms (3A1/3A2/3A3) are widely expressed on a variety of tumors. 1BTN3A1 plays a key role in phosphoantigen activation of Vγ9Vd2 T-cells, key mediators of innate and adaptive anti-tumor immunity. 2Vγ9Vd2 cell infiltration into tumor tissues is associated with a positive prognosis across multiple cancers, 3which makes BTN3A an interesting target for enhancing anti-tumor immunity. ImCheck Therapeutics is developing ICT01, an anti-BTN3A mAb that specifically activates Vγ9Vd2 T-cells. ICT01 is currently in an international, multi-center Phase 1/2a clinical trial (NCT04243499, EVICTION Study). The level of BTN3A expression required for a clinical response to ICT01 is not known. Therefore, we developed novel immunohistochemistry (IHC) methods to enable a precision-medicine based approach to target population selection for dose escalation and potentially guiding patient selection in the expansion cohorts of the ongoing EVICTION study.

Methods A panBTN3A IHC staining that recognizes the three isoforms was developed on fresh frozen (FF) tissues, while BTN3A2- and BTN3A3-specific IHC methods were developed on formalin-fixed paraffin embedded (FFPE) tissues. BTN3A1-specific staining is still under development. Transfected knock-out/knock-in cell lines and positive tissues were used to assess antibody specificity. BTN3A expression was then analyzed on both normal and associated tumor tissue using tissue microarrays (TMA) and selected frozen blocks from tumor biopsies. FACS analyses were also performed on dissociated lung and pancreatic cancer biopsies to determine BTN3A (3 isoforms) membrane expression on tumor-infiltrating immune cells and cancer/stromal cells.

Results In normal tissues, BTN3A2 and BTN3A3 specific IHC signals were granular cytoplasmic in epithelial cells, with positive mononuclear and endothelial cells. Higher expression in lung, colon, and small intestine tissues was observed. Regarding panBTN3A expression, inter-indication and inter-patient heterogeneity was observed among head and neck, lung, melanoma, bladder, colon, pancreas, breast, and prostate cancer tissues, with both cytoplasmic and membranous localizations. The major finding was higher expression of BTN3A2 on malignant cells in melanoma, lung, colon, and prostate cancers, as compared to normal tissue. Finally, FACS analyses of lung and pancreatic cancer tissues revealed stronger expression of all BTN3A isoforms at the cell surface of infiltrated immune cells compared to its expression on stromal cells.

Conclusions These validated IHC methods supported the selection of cancer indications for the EVICTION trial and will potentially help identify specific tumor subtypes and patients that will most likely benefit from ICT01 treatment.

REFERENCES

Molecularly guided multiplexed digital spatial analysis reveals differential gene expression profiles in the WNT-β-catenin pathway between melanoma and prostate tumors

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Background The canonical WNT-β-catenin signaling pathway is vital for development and tissue homeostasis but becomes strongly tumorigenic when dysregulated. Moreover, the transcriptional signature of a cell to promote malignant transformation. However, thorough characterization of these transcriptional signatures has been challenging because traditional methods lack either spatial information, multiplexing, or sensitivity/specificity. To overcome these challenges, we