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1 TUMOUR ASSOCIATED MACROPHAGES IN HPV-RELATED CARCINOMA WITH ADENOID CYSTIC LIKE FEATURES OF THE SINONASAL TRACT: A REVIEW OF THREE CASES

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Background HPV-related carcinoma with adenoid cystic like features of the sinonasal tract is a newly describe entity with histological and immunophenotypic features of surface derived and salivary gland carcinoma. It affects females more than males with age range of 40–70 years and is linked to high risk HPV infection. Most cases present with nasal obstruction and epistaxis. They consist of basaoid cells growing in various sizes separated by fibrocollagenous stroma. It is believed to have a good prognosis. Tumor-associated macrophages (TAMs) are activated macrophages associated with tumor progression in various cancers. TAMs can polarize M1 or M2 type. M1 has a pro-inflammatory function and kills pathogens. Conversely, M2 shows immunosuppressive action and promotes tumor growth. CD68 is known as a pan-macrophage marker. We evaluate the CD68 expression in three cases of HPV-related carcinoma with adenoid cystic like features of the sinonasal tract.

Methods Three cases of HPV-related carcinoma with adenoid cystic like features were retrieved from our archives and stained with p16 and CD68 antibodies. Data was analyzed using spss version 21.

Results Patient ages were 46, 48 and 56 years old respectively, with a female to male ratio of 2:1. Histology showed epithelial surface dysplasia overlying basaoid cell growing in tubular and cribriform patterns. All were strongly positive for p16 stains (figure 1). CD68 showed intratumoral and peritumoral expression in two cases while, one case showed only peritumoral expression. Infiltration of tumor associated macrophages (M2) CD68 cell in this study is associated with increase recurrence of HPV-related adenoid cystic carcinoma of the sinonasal tract (figure 2).

Conclusions The targeting of TAMs in HPV-related adenoid cystic carcinoma of the sinonasal tract and other cancers should be explored in the future using macrophage targeted approach.

Ethics Approval Health research ethics committee ABUTH/ HREC/Y/2017

REFERENCES

2 THE MULTI-PHYSICS AUTOMATED RECONFIGURABLE SEPARATION (MARS®) SYSTEM PROVIDES HIGH PURITY, HIGH RECOVERY AND HIGH THROUGHPUT ENRICHMENT OF IMMUNE CELLS FOR IMMUNOTHERAPY

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Background Immunotherapies have proven to be a potent disruptor of cancer. Large quantities of purified lymphocytes are needed for expansion and downstream manipulation. This purification step has proven to be a major bottleneck for a streamlined cell production process and will only worsen as CAR-T cell therapies move into the clinic. In particular, autologous CAR-T cell therapies directly from cancer patients frequently undergoing existing therapies will require a cell purification technique that provides high recovery, high purity, and high throughput, while being gentle on the cells to ensure downstream efficacy. We present here an integrated system based on multiple physics principles with built-in technologies to achieve cell purification, concentration, and target cell isolation with high recovery and purity at an unprecedented sample flow rate. This platform – the Multi-physics Automated...
Reconfigurable Separation (MARS®) system – combines novel acoustic cell processing and in-flow immuno-magnetic separation technologies with automation of the entire purification workflow for downstream cellular growth, modification, and analysis prior to being administered to patients.

Methods As a fully automated system, the MARS® system can isolate T-cells with high purity from lysed whole blood in as little as 11 minutes with up to 98% recovery and 98% viability without the need for Ficoll or centrifugation. The process is scalable to 10ML of blood, with complete purification requiring 1 hour. The system is designed to fit into a culture hood for sterile cell handling and has been used to isolate T-cells for expansion for downstream T-cell uses.

Results The tunable microfluidic cell processor is a functional module capable of washing and concentrating various sample types including all white blood cell types from whole blood, bone marrow, and apheresis. Additional uses for thawed frozen PBMC, cultured cells and solid tumor dissociation have also been demonstrated. Comparing with conventional centrifugation process, cell preparation by MARS has demonstrated high level of debris removal (>97%), minimal cell loss (>90% recovery) and high cell viability with full automation. MARS is the first-to-market fully automated system to integrate sample preparation and cell isolation into a single platform and is designed to be a versatile tool for downstream cell analysis workflows.

Conclusions MARS is the first to market automatic sample preparation system and is designed to be a versatile tool for downstream cell analysis platforms. The MARS® system is an ideal instrument to prepare CAR-T cells due to its ability to isolate and purify these cells from whole blood with high viability in a completely automated process.

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3 BUTYROPHILIN-3A IS EXPRESSED IN MULTIPLE SOLID TUMORS: TRANSLATIONAL RESEARCH SUPPORTING THE EVICTION STUDY WITH ICT01, AN ANTI-BTN3A MAB ACTIVATING VG9VD2 T-CELLS

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Background Butyrophilin-3A (BTN3A) three isoforms (3A1/3A2/3A3) are widely expressed on a variety of tumors. BTN3A3 plays a key role in phosphoantigen activation ofVG9VD2 T-cells, key mediators of innate and adaptive antitumor immunity. VG9VD2 T-cell infiltration into tumor tissues is associated with a positive prognosis across multiple cancers, which makes BTN3A an interesting target for enhancing antitumor immunity. ImCheck Therapeutics is developing ICT01, an anti-BTN3A mAb that specifically activates VG9VD2 T-cells. ICT01 is currently in an international, multi-center Phase 1a 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 knockout/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 tissue 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.

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4 MOLEULARLY GUIDED MULTIPLEXED DIGITAL SPATIAL ANALYSIS REVEALS DIFFERENTIAL GENE EXPRESSION PROFILES IN THE WNT-/?Catenin PATHWAY BETWEEN MELANOMA AND PROSTATE TUMORS

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Background The canonical WNT-/?-catenin signaling pathway is vital for development and tissue homeostasis but becomes strongly tumorogenic when dysregulated. And alter 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/specifcity. To overcome these challenges, we