00284 In Vitro Modeling of the Tumor Immune Microenvironment in Colorectal Cancer Using Patient-derived Expanded T-cells

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Aims: Colorectal cancer (CRC) is the most common cancer in Singapore. Prognoses for metastatic disease remain poor, driving need for new therapeutic strategies. However, response to immune checkpoint inhibition in CRC is dismal, due to inadequate reactive Tumor Infiltrating Lymphocytes (TILs) and an immunosuppressive tumor microenvironment. Combination therapy using Adoptive Cell Transfer and immune drug modulation is promising, but there are limited experimental models. We aim to expand patient-derived cytotoxic T-cells and develop an ex vivo co-culture system to interrogate pharmacologic or genetic perturbations that modulate anti-cancer immunotoxicity in CRC. We will also demonstrate tumor specificity of expanded T-cells via co-culture with autologous versus allogeneic CRC tumor cell lines.

Methodology: T-cells were cultured from tumor and peripheral blood using IL-2 and engineered auxotrophic K562 aAPC expressing anti-CD3 single-chain antibody, CD86 and 4-1BBL. Phenotype and exhaustion markers (PD-1, Tim-3, CTLA-4 and LAG3) were characterized. Cytotoxic potential was examined by co-culturing with CD3-specific engineered HeLa cells with a Granzyme B FRET reporter system, combined with IFN-γ ELISA of supernatants. Finally, CD8+ T-cells were co-cultured with autologous tumor to identify patient-specific kill.

Result: We successfully expanded and characterized PBMC and TILs from 21 patients, up to 65,000 and 120,000 cumulative fold expansion respectively. TILs had higher PD-1 expression. Expanded T-cells at day 28 were predominantly CD3+ (median 82.8%), with median 12.7% CD8+ T-cells. 14 of 15 patient T-cells demonstrated significant dose and time-dependent cytotoxicity, modifiable by IL-2 and anti-PD-1. Co-culture with autologous versus unmatched allogeneic tumor cells demonstrated significant patient-specific cytotoxicity.

Conclusion: It is feasible to expand T-cells from CRC patients that retain immune-specific cytotoxicity for use in ACT. We also developed an ex vivo patient-specific immunotherapy model to allow further evaluation of immunologic and pharmacologic modulation. Ultimately, this provides a window into translational research to further optimize and develop novel therapeutic strategies for subsequent clinical trials.