Microbiome and Other Environmental Factors

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HARNESSING THE MICROBIOTA TO INCREASE RESPONSE RATES TO IMMUNOTHERAPY

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Background Tumor cell clearance by cytotoxic T lymphocytes (CTL) requires expression of relevant antigens on HLA Class I molecules on the surface of tumor cells. Reduced levels of HLA Class I expression is a common method of immune escape, as it hampers tumor-specific CTLs' ability to detect, recognize, and eliminate tumor cells. Recent data have shown that gut microbiota have a major impact on the clinical response to immune checkpoint inhibitors (ICIs), which could be due to a direct effect on malignant cells. Our hypothesis is that microbiota can influence the immune response by altering HLA Class I expression on tumor cells.

Methods To investigate the ability of bacteria-based products to upregulate HLA Class I expression, we tested two different proprietary microbial derivatives (MDs) on multiple murine and human tumor primary and immortalized cell lines from various tissues, including: breast, myeloid, melanoma, and colon. We next examined if the change in HLA expression was functional by measuring activation levels and cytotoxic capacity of MART-1-specific CTLs following tumor cell treatment with MDs. Lastly, we administered MDs intra-peritoneally in 4T1-bearing Balb/c mice to sensitize 4T1 tumors to combination treatment with anti-PD-1 ICI.

Results Our results to date show that *in vitro* treatment with MDs can upregulate surface HLA, albeit not uniformly across all tumor types, with breast and myeloid tumor cells showing the largest increase across the cell lines tested (figure 1). The MD-dependent HLA increase subsequently boosted CTL recognition of tumor cells without increasing background reactivity. The increased CTL degranulation correlated to the tumor cells' increased surface HLA expression and was consistent whether the antigen was endogenous (5% increase, $p < 0.0001$, figure 2A) or added exogenously (15–30% increase, $p < 0.01$ and $p < 0.0001$ figure 2B). In combination with anti-PD-1 *in vivo*, MD treatment significantly abrogated tumor growth when compared to anti-PD-1 combined with the vehicle control ($p < 0.0001$, figure 3A) and tumors harvested from MD-treated mice expressed higher levels of MHC Class I compared to the vehicle control cohort ($p < 0.05$, figure 3B). Additionally, splenocytes from MD-treated mice showed increased recognition of 4T1 tumor cells when re-challenged *in vitro* (10% increase in $CD8^+41BB^+$ cells, $p < 0.0001$, figure 3C).

Abstract 835 Figure 1 Class I surface expression after MD treatment. (A) breast (B) colon (C) melanoma and (D) myeloid human cancer cell

lines were incubated with 5 (light bars) or 10 (dark bars) mg/mL MD#1, MD#2, and 10 mg/mL respective vehicle control (empty bars). (E) and (F) were treated with 10 mg/mL (dark bars) MD#1, MD#2, or respective vehicle controls (empty bars). After 48 hours, HLA Class I (A-D), H-2k^b (E), and H-2k^d (F) surface expression was measured by flow cytometry. Experiments repeated at least in duplicate. Statistical analysis by 2-way ANOVA, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Abstract 835 Figure 2 Antigen-specific CTL activation. Tumor cells were pre-treated for 48 hours with 10 mg/mL vehicle or MD, then washed and co-cultured for 5 hours with MART-1 specific CTL. A) primary HLA-A2⁺ melanoma cells that are negative (Mel12) or positive (Mel13) for the MART-1 antigen, and B) Thp1 loaded or not with MART-1 peptide. $CD8^+CD107a^+$ cells measured by flow cytometry. Experiments repeated in triplicate, statistical analysis by two-way ANOVA.

Abstract 835 Figure 3 *In vivo* treatment with MD. Fifteen 6-week-old Balb/c mice were subcutaneously inoculated with 1.5×10^5 4T1 tumor cells and divided into 3 treatment groups on day 3 based on equivalent tumor size. Mice were treated with 250 μ g microbial derivatives (MD#1) or vehicle control (vehicle #1) in combination with anti-PD-1 (200 μ g; clone 29F.1A12) starting on day 3 and continued every other day for a total of 4 injections (black arrows). (A) Tumor measurements were taken every other day using a caliper and volume calculated using the formula: tumor volume = (length $\times$ width²) $\div$ 2 (B) 2×10^5 splenocytes were co-cultured 1:1 with 4T1 tumor cells *in vitro* and T cell activation (percent $CD8^+41BB^+$) was measured by flow cytometry. Experiment repeated in duplicate, statistical analysis by 2-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Conclusions Our results thus far confirm that our proprietary MDs can increase HLA expression on tumor cells, and that this can lead to increased recognition by antigen-specific CTL both *in vitro* and *in vivo*. This suggests that MDs could be explored in combination with ICIs to enhance clinical anti-cancer immune responses.

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