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POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

Pegylated Long-Acting Interferon Gamma Treatment Enhances MHC Class II Expression and the Graft-Versus-Leukemia Effect in Preclinical Models of AML Relapse

Matt Christopher, MD[1], *Vaishali Basu, PhD*[1], *Gregory Mannino*[1]

[1]Washington University School of Medicine, St. Louis„ MO

Relapse after allogeneic hematopoietic stem cell transplantation (HCT)represents the most common type of treatment failure and is thought to result in part from AML cell escape from immune pressure exerted by donor immune cells (the "Graft-versus-leukemia" effect, GvL). We and others have previously reported downregulation of MHC class II (MHC-II) expression in up to half of AML cases relapsing after transplantation, but it is unclear whether a) loss of MHC-II contributes mechanistically to loss of GvL, and b) whether reversal of MHC-II downregulation can restore GvL. We tested the role of MHC-II in mediating GvL in a mouse model of minor-antigen mismatch HCT where C57BL/6 mice (B6) bearing syngeneic AML cells (H-2b) were co-transplanted with or without CD3+ T cells from C3.SW-H2b/SnJ mice (H-2b). Since murine AML cells do not generally express MHC-II at baseline, we transduced MLL-AF9-driven B6 AML cells with a fluorescently-tagged retrovirus expressing *CIITA* the main transcriptional regulator of MHC-II genes. MHC-II-expressing AML cells were selectively deleted when co-transplanted with donor T cells, in contrast to animals that received no T cells (Figure 1, right) or mice that received AML cells transduced with empty vector (Figure 1, left). To extend these findings to human cells, we used both MHC-II blocking antibodies and CRISPR-mediated disruption of *CIITA* in human AML cell lines and primary samples. Loss or blocking of MHC-II led to diminished the ability of AML cells to stimulate MHC-mismatched donor T cells in a mixed lymphocyte reaction.
Loss of MHC-II expression in AML cells can be reversed *in vitro* by Interferon gamma (IFNG). We tested the ability of IFNG to enhance the GvL by culturing AML cell lines and primary patient samples with or without IFNG for 48 hours. IFNG-treatment enhanced the ability of AML cells from some-but not all-samples to stimulate third-party T cells. Interestingly, enhanced stimulation was observed in both CD4 and CD8 cells, suggesting that IFNG may also potentiate GvL through MHC-II-independent pathways. Since the use of IFNG *in vivo* may be limited by its short half-life, we tested a novel long-acting pegylated human interferon gamma (peg-IFNG, Bolder BioTechnology) in a xenograft model of AML. Immunodeficient mice were engrafted with MHC-II-low human AML cells. After engraftment, mice (n=3 each group) received six doses of IFNG, peg-IFNG, or vehicle (15mcg given s.c. M-W-F). After treatment, mice were sacrificed and MHC-II expression was quantified on human AML cells isolated from bone marrow of treated mice. IFNG treatment increased MHC-II expression modestly compared to vehicle (2.7-fold, p=0.04), while peg-IFNG much more robustly stimulated MHC-II stimulation on human AML cells in this xenograft model (12-fold, p=0.02).
Finally, we tested whether peg-IFNG can enhance AML cell clearance in a xenograft model. Luciferin-labelled THP1 cells (low MHC-II expression) were injected on day 0 into immundeficient mice (n=8-9 each group). On day 7, mice started treatment with peg-IFNG or vehicle (15mcg given s.c. M-W-F for two weeks). On day 21, baseline tumor burden was measured using bioluminescence, and healthy third-party donor T cells were injected. Mice were followed with weekly CBC and bioluminescence measurments, and moribund mice were sacrificed and banked. Mice treated with peg-IFNG plus T cells had decreased tumor burden during the course of the experiment compared to untreated controls as well as mice treated with T cells plus vehicle only (Figure 2, $p<0.01$ by ANOVA for both comparisons). In addition, mice treated with peg-IFNG had significantly improved survival compared to control groups (median survival 79 days compared to 63 days and 67 days, $p<0.05$ by Log-Rank test). Together, these findings suggest that long-acting IFNG analogues may effectively enhance GvL in AML patients after HCT.

Disclosures No relevant conflicts of interest to declare.

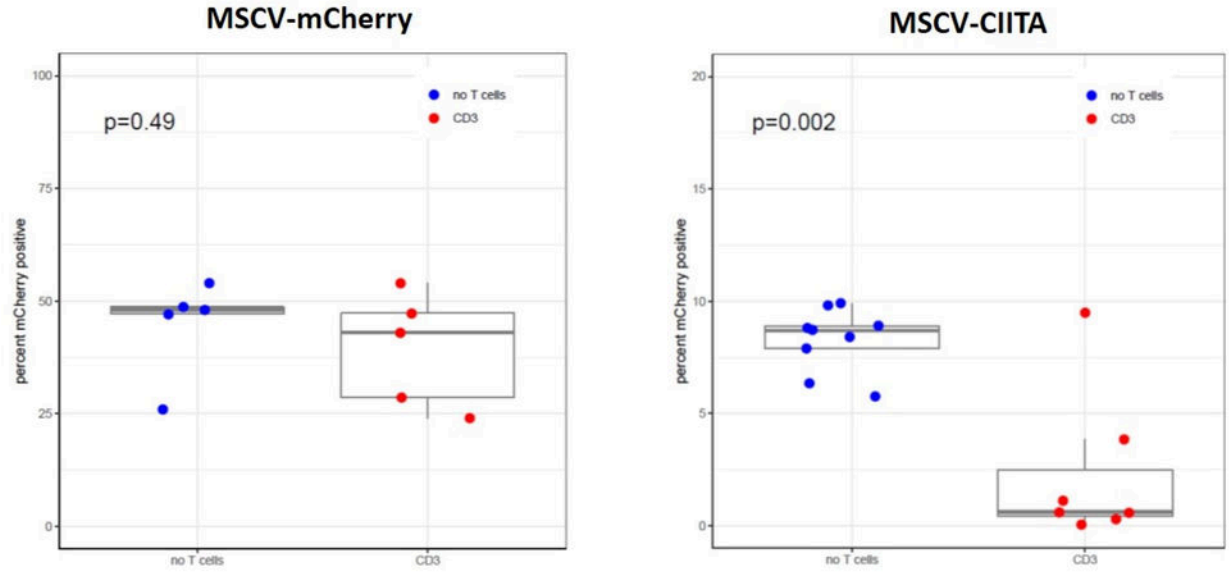

Figure 1

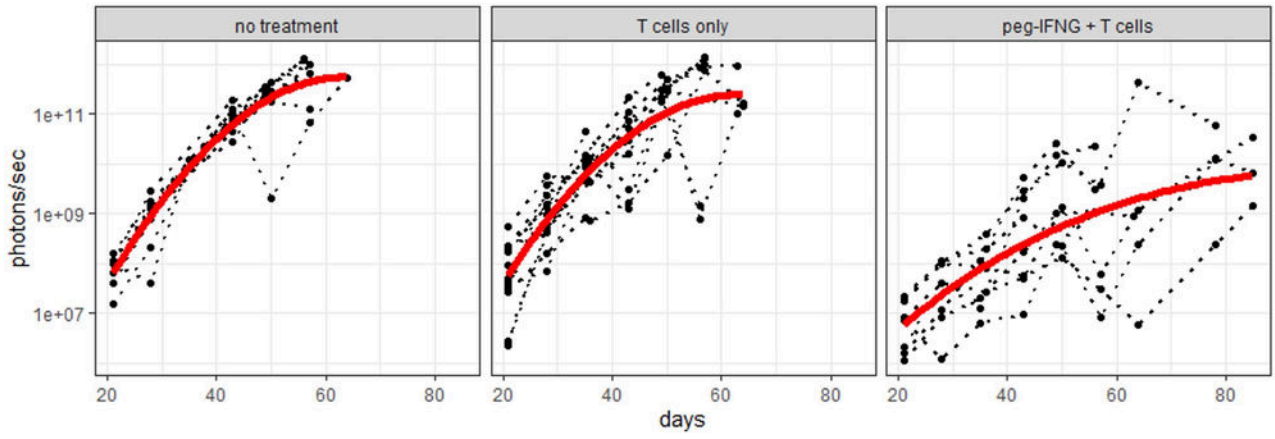

Figure 2

Figure 1

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