



Figure 2. Fold-change in pCCR5 on CD8 T-cells in response to CCL4 stimulation with or without MVC

unstimulated control. The surface expression of CCR5 was measured separately by flow cytometry.

Results: Twenty-four reduced-intensity alloSCT recipients received MVC 300 mg b.i.d. orally from day -3 to day +90 on an ongoing phase II study. Recipient blood was tested on days -6 (before MVC), 0 and 14, and donor blood was tested on day 0. We observed significant variability in surface CCR5 expression on T-cells in donors (range 0–9%) and recipients (range 0–49%). Recipient surface CCR5 expression increased from d-6 to d0 on both CD4 (mean 3.6 to 7.3%, $p=0.005$) and CD8 (mean 13.5 to 30.6%, $p=0.005$) T-cells, consistent with blockade of receptor internalization.

CD8 T-cell pCCR5 levels increased in response to CCL4 stimulation on d -6 ($p=0.01$) and decreased in response to MVC ($p=0.002$), compared to unstimulated blood (Fig. 2). A decrease in pCCR5 levels in response to MVC was observed even without CCL4 stimulation, reflecting loss of background CCR5 activity ($p=0.0002$). In contrast, d0 pCCR5 levels failed to increase in response to CCL4 ($p=0.32$), nor did they decrease in response to additional MVC ($p=0.13$), mirroring effective *in vivo* blockade of CCR5.

The magnitude of increase in CD8 T-cell pCCR5 levels in response to CCL4 stimulation on d-6 correlated with CCR5 surface expression ($r=0.55$, $p=0.02$). The same was true of CD4 T-cells ($r=0.76$, $p=0.0004$). On d0, however, the ability to stimulate CCR5 inversely correlated with CCR5 surface expression for both CD8 ($r=-0.46$, $p=0.04$) and CD4 ($r=-0.62$, $p=0.004$), suggesting that greater CCR5 surface upregulation may serve as an indicator for complete receptor blockade.

Conclusions: Intracellular phosphorylation of CCR5 in response to stimulation is efficiently blocked by MVC in patients undergoing alloSCT, as measured by a novel phosphoflow assay. Upregulation of surface CCR5 may indicate effective CCR5 blockade and should be explored as a biomarker for drug response.

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CD4+CD25+ Invariant Natural Killer T Cells Are Enriched in Cord Blood: Implication on Immune-Regulation

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CD1d-restricted invariant Natural Killer (iNK) T cells are rare but powerful regulatory T cells that can produce a large amount of both inflammatory (Th-1 type) and regulatory (Th-2 type) cytokines upon activation. Therefore, they can potentially shape subsequent adaptive immune responses towards inflammation or immune tolerance. Unlike the other regulatory immune cells, iNK T cells are heterogeneous in phenotype (CD4+ vs CD4- vs CD8a+), and display a spectrum of function ranging from regulatory to inflammatory responses. Given the potential regulatory function, iNKT cells are thought to play a role in preventing Graft Versus Host Disease (GVHD) in allogeneic stem cell transplant (SCT). Yet, the precise mechanism of immune regulation by iNKT cells in allogeneic SCT setting has not been clearly elucidated. As the cord blood stem cell transplantation is known to have a reduced incidence of GVHD as compared to matched adult donor allogeneic SCT, we hypothesize that iNK T cells in cord blood possess the regulatory phenotype and function as compared to those in healthy adults, thus play a role in preventing GVHD. To test this hypothesis, we first characterized the phenotype of iNK T cells from 25 cord blood and 25 peripheral blood of healthy adults using multi-color Flow Cytometry. We found that iNK T cells were present in variable frequencies ranging from 0.01% to 2% of T cells from healthy adults but rather in similar frequencies around 0.1% of T cells from cord blood. Interestingly, the extremely high percentage of CD4+CD25+CD161- iNK T cells was present in cord blood, while iNK T cells from healthy adults were heterogeneous in phenotype with a trend towards higher percentage of CD4-CD25-CD161+ iNK T cells. Next, we investigated whether CD4+CD25+ iNK T cells from cord blood indeed display regulatory property, and assessed cytokine production profile of *ex vivo* expanded polyclonal iNK T cells stimulated by autologous or allogeneic dendritic cells in the presence of alpha-GalCer, agonist glycolipid antigen. We found that the iNK T cells from cord blood preferentially produced Th-2 type cytokines (GM-CSF, IL-13, IL-4, IL-5) as compared to iNK T cells from healthy adults. Lastly, polyclonal iNK T cells showed significant cytokine production to autologous or allogeneic dendritic cells in the absence of alpha-GalCer, suggesting that iNK T cells can be activated by endogenous glycolipid antigens in physiologic condition. In summary, we demonstrated that CD4+CD25+CD161-iNK T cells were enriched in cord blood, and indeed displayed regulatory property. Our results suggest that CD4+CD25+ iNK T cells may play role in immunoregulation to prevent GVHD via regulatory function.

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The MEK Inhibitor Trametinib Selectively Suppresses GVHD, While Sparing GVT Effects

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Background and Objective: Antineoplastic MEK inhibitors have immunomodulatory activity. MEK inhibition selectively suppresses naïve/central memory T cells while sparing effector memory T cells, and delays the onset of murine GVHD (Shindo, Kim, Komanduri et al. *Blood* 2013). We investigated whether MEK inhibition selectively suppresses