CD33-Deleted Hematopoietic Cells (Trem-cel) Are Protected From CD33xCD3 Bispecific Antibody Treatment And Produce Significantly Reduced Levels Of Inflammatory Cytokines In Preclinical Studies

#3425

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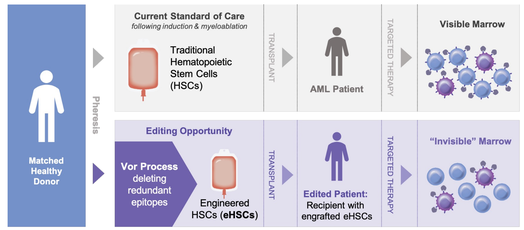
INTRODUCTION

- Though allogeneic hematopoietic cell transplantation (alloHCT) is a potentially curative therapy for patients with high-risk acute myeloid leukemia (AML), post-HCT relapse occurs in up to 70% of these patients[1].This highlights the need for new therapeutic approaches; however, a lack of cancer specific targets has hampered effective targeted therapy.
- One described AML target is CD33; however, CD33-directed therapies have been challenged by cytopenias due to on-target, off-tumor toxicity and, in the case of bispecific antibodies, cytokine release syndrome (CRS), due to the abundance of CD33 antigen.
- Tremtelectogene empogeditemcel (trem-cel) is a gene-edited human hematopoietic stem and progenitor cell (hHSPC) transplant product in phase 1 clinical trial (NCT04849910). Trem-cel is generated using dual mobilized CD34$^+$ HSPCs in which the biologically dispensable CD33 is removed to enable exclusive CD33 targeting on AML cells.
- Pairing trem-cel with JNJ-67571244[2], a CD33 targeting bispecific antibody (BiSAb), is an attractive approach because trem-cel may provide not only heme protection but also reduce CRS due to less CD33 antigen, ultimately improving post HCT outcomes in AML patients.

Figure 1: Vor Bio's Treatment System Approach



OBJECTIVES

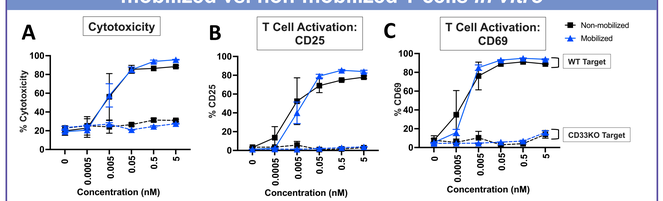
- Demonstrate that CD33KO (knockout) cells (trem-cel) are protected from CD33 specific killing by the CD33 targeting bispecific antibody JNJ-67571244 in both *in vitro* and *in vivo* preclinical studies.
- Demonstrate that trem-cel human HSPC xenotransplanted into mice produce reduced levels of inflammatory cytokines associated with CRS when treated with JNJ-67571244.

METHODS

- *In vitro* and *in vivo* proof of concept (POC) experiments were performed using CD34$^+$ hHSPCs that were edited using either CRISPR/Cas9 to delete *CD33* (trem-cel) or treated with a non-targeting guide RNA (gRNA) control (gCtrl).
- Monocytes were differentiated *in vitro* (MIVD) from either trem-cel or gCtrl hHSPCs and assayed for editing frequency, CD33 protein expression and myeloid phenotype by flow cytometry before addition into *in vitro* cytotoxicity assays.
- *In vitro* cytotoxicity assays were carried out by co-culturing target cells, either AML cell lines or MIVD cells, with T cells and treated with JNJ-67571244 at various concentrations. Cytotoxicity was analyzed using flow cytometry at 48hr and 72hr and cytokine concentrations in the supernatants were measured using Luminex.
- *In vivo* xenotransplant studies were performed by engrafting trem-cel or gCtrl hHSPCs into NSG-SGM3 mice followed by treatment with either control bispecific (CD3xNull) or JNJ-67571244. Plasma cytokine concentration were measured via Luminex and hematopoietic tissues were analyzed by flow cytometry to measure protection from JNJ-67571244 cytotoxic killing.

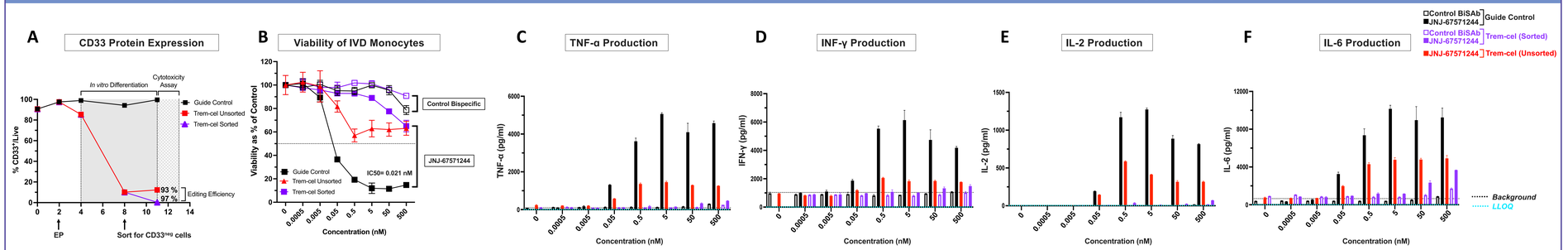
RESULTS

Figure 2. Comparable JNJ-67571244 efficacy between dual mobilized vs. non-mobilized T cells *in vitro*



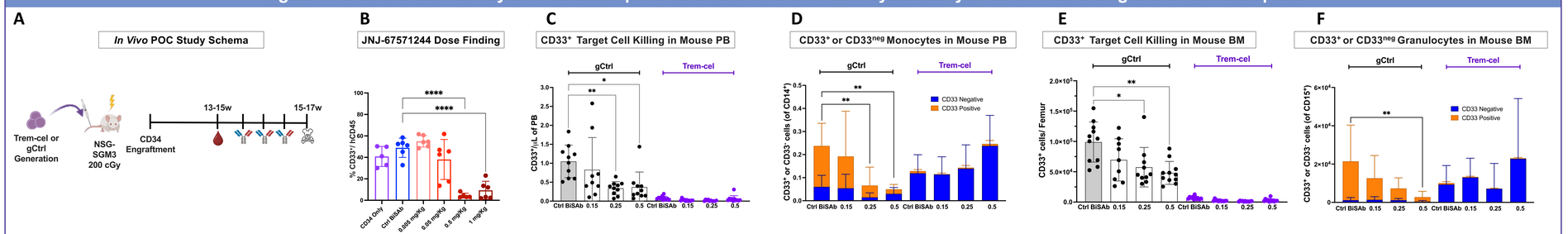
Target AML cell line MOLM-13 or CD33KO MOLM-13 were co-cultured with T cells from either dual mobilized or non-mobilized sources and treated with JNJ-67571244 *in vitro*. This was to determine whether mobilized T cells perform similarly to non-mobilized in the context of bispecific treatment as dual mobilized T cells may be considered for use in combination with trem-cel treatment system and the following studies. **(A)** Cytotoxicity as measured by the percentage of dead cells (Annexin V$^+$ and Live/Dead stain$^+$), and T cell activation (CD25 %) **(B)** and (CD69 %) **(C)** were similar between mobilized or non-mobilized T cell sources. N=1 donor. Data shown as mean ± standard deviation.

Figure 3. Trem-cel-derived monocytes are protected from JNJ-67571244 cytotoxicity *in vitro* and demonstrate decreased cytokine production in co-cultures



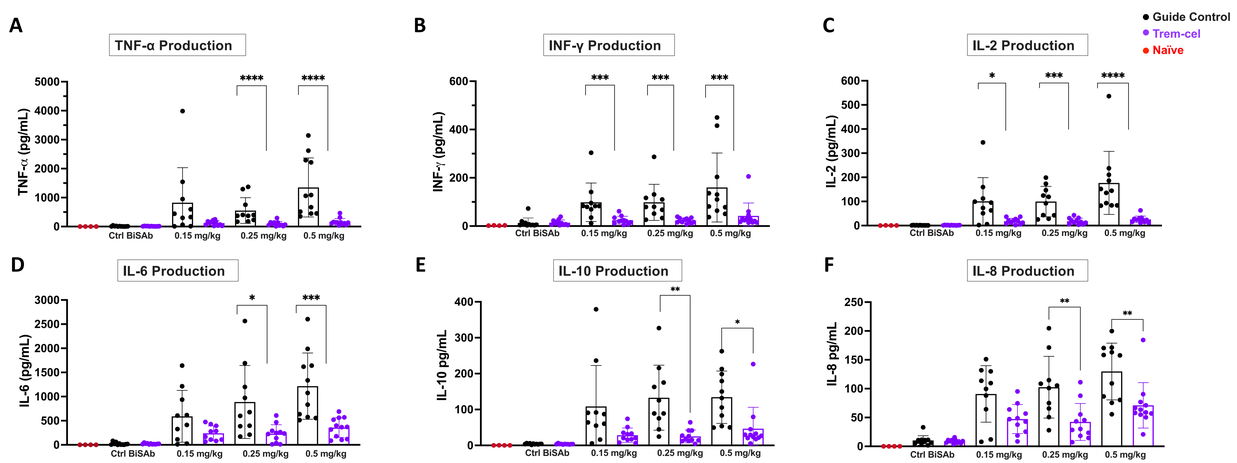
Dual mobilized peripheral blood CD34$^+$ hHSPCs were *in vitro* differentiated into monocytes after CD33 editing. Trem-cel MIVD were then sorted into CD33neg cells and subjected to a cytotoxicity assay. **(A)** High CD33 editing and CD33 protein KO was maintained throughout differentiation. **(B)** MIVD cells, either gCtrl, trem-cel or sorted trem-cel were co-cultured with donor matched T cells as well as either JNJ-67571244 or control bispecific (CD3xnull) at increasing concentrations. Viability was assessed using flow cytometry and expressed as a percentage of control which was MIVD + T cells (0 nM Control BiSAb or JNJ 67571244). IC_{50} Cannot be accurately calculated for ▲ or ■. Cytokine production in co-cultures **(C)** TNF-α, **(D)** INF-γ,**(E)** IL-2 and **(F)** IL-6. IL-8 and IL-10 (data not shown) also showed similar results. Background: Cytokines from MIVD cells + T cells alone. N=1 donor. LLOQ: lower than limit of quantification. Data shown as mean ± standard deviation.

Figure 4. Trem-cel-derived myeloid cells are protected from JNJ-67571244 cytotoxicity in CD34$^+$ HSPC engrafted xenotransplant



(A) Schematic of mouse xenotransplant study and JNJ-67571244 dosing. **(B)** A JNJ-67571244 dose finding study was performed in CD34$^+$ HSPC engrafted NSG-SGM3 mice to determine optimum dose for targeting normal CD33$^+$ hematopoietic cells *in vivo*. After three doses of JNJ-67571244, trem-cel or gCtrl engrafted mouse tissues were harvested and flow cytometry performed to determine efficacy of JNJ-67571244 on CD33$^+$ cells and protection of trem-cel-derived cells. **(C)** gCtrl-derived human CD33$^+$ cells in the peripheral blood (PB) were killed in a dose dependent manner by JNJ-67571244 as shown by CD33$^+$ cell counts/ µL of PB. **(D)** gCtrl-derived CD14$^+$ monocytes, which are largely CD33$^+$, are effectively killed by JNJ-67571244; however, trem-cel-derived monocytes maintain cell counts suggesting they are protected from JNJ-67571244 treatment. **(E)** gCtrl-derived human CD33$^+$ cell counts per femur decreased with JNJ-67571244 treatment. **(F)** gCtrl-derived CD15$^+$ granulocytes, which are largely CD33$^+$, are effectively killed by JNJ-67571244 in the bone marrow (BM), whilst trem-cel-derived granulocytes maintain cell counts per femur. N=1 donor shown, however data from another donor is similar (Data not shown). Data shown as mean ± standard deviation. *p<0.05, **p≤ 0.01, ****p<0.0001.

Figure 5 . Reduced cytokine released from JNJ-67571244 treated Trem-cel engrafted mice



Trem-cel or gCtrl engrafted mice were dosed with either JNJ-67571244 or control bispecific and two hours later plasma was collected to examine cytokine production via Luminex. CRS cytokines examined include **(A)** TNF- α, **(B)** INF-γ,**(C)** Il-2, **(D)** IL-6 **(E)** IL-10 and **(F)** IL-8. trem-cel mice treated with JNJ-67571244 produced significantly less cytokines compared to the gCtrl engrafted mice as trem-cel-derived cells were not targeted by JNJ-67571244. Control Bispecific was dosed at 0.5 mg/kg. Mice not engrafted with CD34$^+$ hHSPCs were termed naïve and used a negative controls. N=1 donor shown; however, data from another donor is similar (Data not shown). Data shown as mean ± standard deviation. *p<0.05, **p≤ 0.01, ****p<0.0001.

Abbreviations: Acute Myeloid Leukemia (AML); allogenic hematopoietic stem cell transplant (alloHCT); cytokine release syndrome (CRS); Tremtelectogene empogeditemcel (trem-cel); human hematopoietic stem and progenitor cell (HSPC); bispecific antibody (BiSAb); KO (knockout); CD33KO cells (trem-cel); *in vitro* differentiated monocytes (MIVD); guide RNA (gRNA); electroporation (EP); peripheral blood (PB); bone marrow (BM); proof of concept (POC)

CONCLUSIONS

- Dual mobilized T cells can kill targets similarly as non-mobilized T cells in the presence of JNJ-67571244 *in vitro*.
- Trem-cel-derived *in vitro* differentiated monocytes treated with JNJ-67571244 are more viable in cytotoxicity assays compared to control suggesting they are protected from JNJ-67571244 treatment *in vitro*.
- Trem-cel-derived *in vitro* differentiated monocytes and T cells produce significantly less inflammatory cytokines upon JNJ-67571244 treatment as they are protected from cytotoxic killing unlike guide control cells.
- Trem-cel-derived monocytes and granulocytes are protected from CD33$^+$ targeting by JNJ-67571244 *in vivo*.
- Trem-cel engrafted mice treated with JNJ-67571244 produce significantly less cytokines in mouse plasma as a result of protection from JNJ-67571244 killing.
- These findings enable the development of a next-generation AML treatment strategy by pairing a trem-cel transplant with a subsequent CD33-directed modality such as bispecific antibodies or CAR-T cells to potentially improve safety and efficacy while minimizing myelotoxicity and CRS.

Acknowledgments

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References

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