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POSTER ABSTRACTS

703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Enhanced T Cell Function of PTPN2 Deleted CAR-T Cells Comes at a Cost: PTPN2 Knockout CAR-Ts Secrete More Cytokines and Demonstrate Increased Cytotoxicity, but Exhibit More Severe CRS and Icans in a Non-Human Primate Model

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Background: Despite the success of CD19 CAR-T cells (CAR-Ts) in inducing remission, relapse remains a major issue. Previous studies have shown that upregulation of negative T cell regulators can contribute to CAR-T failure, and that deletion of these regulators can enhance CAR-T efficacy. A potent negative regulator is PTPN2, which inhibits T cell function by modulating T cell receptor and cytokine signaling pathways. While murine models have shown that *PTPN2*-knockout T cells improve tumor control, these models fail to reliably predict clinical efficacy or toxicity. To improve this predictability, we investigated *PTPN2* KO CAR-Ts in primary human cells and in a NHP model of B-cell directed CAR-T therapy.
Methods: CRISPR/Cas9-mediated *PTPN2* deleted ('KO') human CD19 CAR-Ts were assessed for phenotype/function using cytokine secretion and cytotoxicity assays. *PTPN2* KO NHP CD20 CAR-Ts were administered to lymphodepleted NHPs using our established model and a dose escalation with Level 1: $6x10^4$, Level 2: $6x10^5$, Level 3: $3x10^6$, and Level 4: $6x10^6$ CAR-T/kg, followed by assessment for CAR-T expansion, B cell aplasia, CRS and ICANS.
Results: *PTPN2* KO was successfully achieved in human CAR-Ts (83.8 + 5.2% deletion). IFNγ production was significantly higher in *PTPN2* KO CD19 CAR-Ts vs control cells (35.1 + 0.97% vs 23.4 + 0.98%, p=0.001). In cytotoxicity assays targeting the B-ALL cell line NALM6, *PTPN2* KO CD19 CAR-Ts demonstrated significantly increased killing vs control cells (for example, at E:T ratio 1:1 59.6 + 9.8% vs 42.5 + 12.9% p = 0.03).
We evaluated the proliferation, efficacy, and toxicity of *PTPN2* KO CD20 CAR-Ts in 5 NHPs. The CAR-T *PTPN2* KO rate ranged from 63-91%. During dose escalation, no expansion of CAR-Ts was observed at dose levels 1 and 2 (n=1 for each, **Fig 1**). However, all animals infused at dose level 3 (n=3, $3x10^6$ CAR-Ts/kg) demonstrated CAR-T expansion (max expansion 18.6 + 6.8% CAR-Ts) and B cell aplasia, and all animals developed laboratory and clinical signs of CRS and ICANS (**Fig 1**). One animal at dose level 3 experienced CRS on day +4 that was severe enough to require tocilizumab, with symptoms subsequently improving. On day +6, this animal also developed ICANS, including ataxia and muscle weakness, requiring 1 dose of Dexamethasone (1mg/kg), after which symptoms resolved.
One animal received dose level 4 ($6x10^6$ CAR-Ts/kg). This recipient displayed earlier and higher expansion of CAR-Ts (day 3: 16.7%, day 7: 56.3% CAR-Ts, **Fig.1**), and developed severe CRS (with elevated CRP, LDH (not shown) and Ferritin, **Fig.1**) and ICANS that were unresponsive to treatment with 2 doses of tocilizumab and multiple doses of dexamethasone, with this recipient meeting humane euthanasia endpoints. Importantly, previous animals (n=5) receiving 6-12 x 10^6 *PTPN2* expressing (WT) CAR-Ts/kg, did not develop severe CRS nor ICANS (and did not require Toci or Dex). This suggests that *PTPN2* KO CAR-Ts are associated with augmented toxicities.
An extensive terminal analysis of the Dose Level 4 animal was performed; pathology demonstrated widespread cerebral edema and CAR-T CNS infiltration. Because the CAR-T infusion contained both *PTPN2* KO and *PTPN2* WT CAR-Ts, an assessment could be made for enrichment of *PTPN2* KO CAR-Ts in the blood and brain parenchyma. Compared to the infused product (63% KO CAR-Ts, **Fig.2)**, there was enrichment for KO CAR-Ts in the blood (72% KO CAR-Ts) and the brain parenchyma (84% KO CAR-Ts, **Fig 2**). This suggests increased expansion and CNS infiltration of *PTPN2* KO versus *PTPN2* WT CAR-Ts. Flow cytometry analysis of the *PTPN2* KO CAR-Ts from the blood at maximum expansion revealed high expression of the prolifera-

tion and activation markers Ki67 and GZMB (84.6%, 74.5% of all CAR-Ts). Of note, at Dose Level 3, neither CAR-T persistence (15 + 2 days vs 20 + 5 days) nor duration of B cell aplasia (41+ 5 days vs 37 + 3 days) were increased with *PTPN2* KO CAR-Ts vs historic controls infused with *PTPN2* WT CAR-Ts.

Conclusion: We demonstrate that PTPN2 KO CAR-T cells possess increased effector function, proliferation, and CNS infiltration versus PTPN2 WT CAR-Ts. However, this enhanced efficacy was associated with increased severity of CRS and ICANS, emphasizing that careful attention to the dose of PTPN2 KO CAR-Ts would be important. These results also highlight the utility of evaluating gene-modified CAR-Ts in clinically relevant animal models, to thoroughly assess their efficacy and toxicity profiles.

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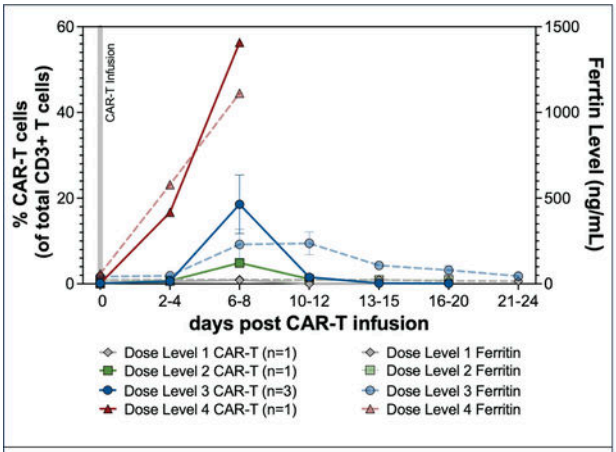

Figure 1: Dose escalation study of PTPN2 KO CD20 CAR-T cells in NHP: Higher CAR-T doses result in greater CAR-T expansion and more severe CRS.
Expansion of escalating doses of PTPN2 KO CD20 CAR-Ts in the peripheral blood after infusion, as measured by the % CAR-Ts compared to total CD3+ T cells (Left axis). Also shown is the serum Ferritin level, as a representative CRS marker (Right axis). No CAR-T expansion and no Ferritin elevation were seen at dose level 1 ($6x10^4$/kg, Gray traces) or at dose level 2 ($6x10^5$/kg, Green traces). Concurrent expansion of CAR-Ts and elevation of Ferritin were observed at dose level 3 ($3x10^6$/kg, n=3, Blue traces). The highest CAR-T expansion and Ferritin levels were observed at dose level 4 ($6x10^6$/kg, Red traces).

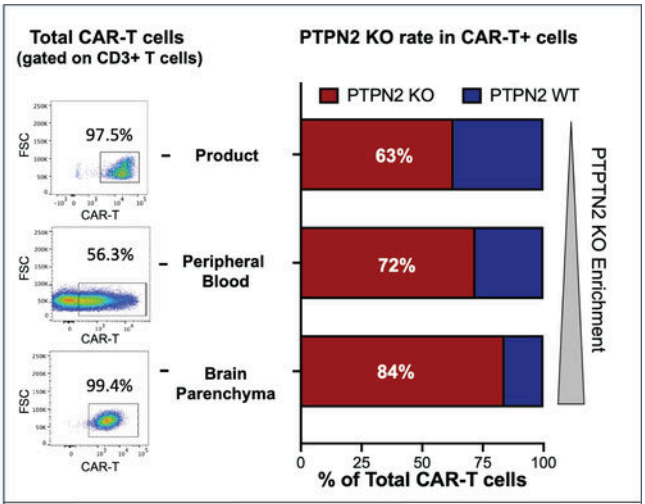

Figure 2: Enrichment of PTPN2 KO CD20 CAR-T cells in the CNS and the blood compared to the infused product. The PTPN2 KO rate was determined by sequencing of flow cytometrically sorted CAR-Ts obtained from the infused CAR-T product, the blood, and the brain parenchyma at terminal analysis in the Dose Level 4 recipient. The percentage of PTPN2 KO CAR-Ts compared to total CAR-Ts demonstrates a progressive enrichment in the CNS parenchyma > peripheral blood > infused product.

Figure 1

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