2005

Effective Cell Dose and Functional Attributes of Azercabtagene Zapreleucel (Azer-cel; PBCAR0191) Associated with Allogeneic CAR T-Cell Safety and Efficacy in Patients with Relapsed/Refractory B-Cell Lymphoma

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INTRODUCTION

- Autologous (Auto) Chimeric Antigen Receptor (CAR) T cell therapy remains one of the most promising approaches in the treatment of hematological malignancies. However, 30-60% of patients relapse after treatment and represent a growing population with high unmet need.
- The post-auto CAR T market is expected to grow significantly with advancement of auto CAR T therapies into the second line DLBCL setting. Ready access to an allogeneic (allo), healthy donor-derived, off-the-shelf CAR T cryopreserved product has the potential to significantly improve outcomes for this fragile, auto-relapsed patient population.
- Azercabtagene Zapreleucel (Azer-cel; PBCAR0191) is an investigational anti-CD19 allogeneic CAR T candidate being evaluated in a Phase 1/2a clinical trial (NCT03666000) of adult subjects with relapsed or refractory (R/R) non-Hodgkin lymphoma, including patients with aggressive NHL subtypes such as DLBCL lymphoma that have relapsed following prior autologous CAR T treatment.
- Characterization of product cellular attributes contributing to the *in vivo* expansion and toxicity of allogeneic, CD19-directed CAR T therapy is necessary to optimize efficacy and safety. In this phase 1/2a study of the allogeneic, CD19-directed CAR T, Azer-cel, we analyzed post-thaw product attributes and cell composition of the infused product associated with pharmacokinetics (PK), pharmacodynamics (PD), and clinical outcomes.

METHODS

- Azer-cel, an allogeneic, CD19-directed cellular therapy was derived from healthy donor cells.
  - Created with a single-step ARCUS gene editing process minimizing translocations and off-target editing.
  - Graft-versus-host disease (GvHD) is mitigated by ARCUS knock out of T-cell receptor alpha chain (TRAC) gene.
- Azer-cel was administered as a single infusion to 44 subjects with non-Hodgkin's lymphoma (NHL) across several dose levels and fludarabine/cyclophosphamide lymphodepletion regimens.
- CAR T cell peak expansion (C_{max}), and area under the concentration-time curve (AUC) were assessed by flow cytometry. Pharmacodynamic response was assessed using multiplex cytokine assays.
- Azer-cel post-thaw cell composition was assessed by flow cytometry to distinguish the following:
  - Stem cell memory (SCM)/central memory (CM) and less differentiated CAR T cell population ($CCR7^+$).
  - Effector memory/effectors and more differentiated CAR T cell population ($CCR7^-$).
- Effective CAR T cell dose was calculated for each patient based on their infusion volume and the post-thaw concentration of non-apoptotic $CCR7^+$ CAR T cells/mL, using flow cytometry.
- Overall tumor burden was measured as sum of the products of perpendicular diameters of target lesions and response was evaluated using Lugano 2016 criteria. Responders had either a complete or partial response at Day 28 or later.
- Relationships of product attributes to safety events, PK/PD responses and efficacy were assessed using univariate correlative analyses (variables selected based on Pearson correlation coefficients ρ(rho)≥0.35 unless specified and p value ≤0.05).

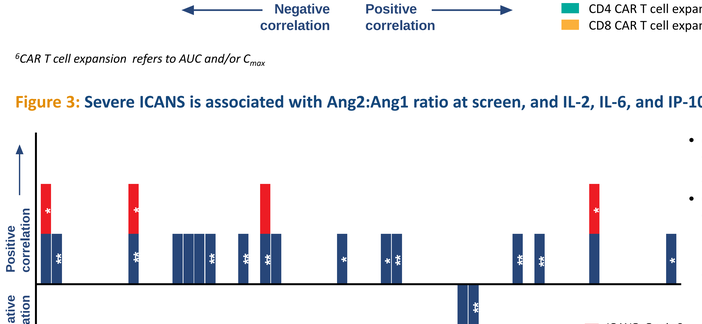
Table 1. Study overview and patient baseline data

Overall		N=44
# Dose levels (DL)		4[1]
# Lymphodepletion (LD) regimens		3[2]
# sites		12
Patient population age (y), median (range)		62 (34-81)
Refractory, n (%)		13 (30%)
Subtype diagnosis, n (%)	*Diffuse Large B-cell Lymphoma*	21(48%)
	CLL with Richter's transformation	6(14%)
	Follicular lymphoma	5(11%)
	Mantle Cell Lymphoma	5(11%)
	Transformed follicular lymphoma	4(9%)
	High Grade B-cell lymphoma	2(5%)
	Small Lymphocytic Lymphoma	1(2%)
# **prior treatments,** median (range)		4 (1-11)
# **Number of autologous CAR T experienced**		15 (34%)

[1] *DL1- 3 x 10^5 CAR T cells/kg, DL2- 1 x 10^6 CAR T cells/kg, DL3a- 3 x 10^6 CAR T cells/kg, DL4b- 500 x 10^6 CAR T cells/flat dose.*
[2] *Standard LD: Fludarabine - 30/mg/m^2/d (3d) + Cyclophosphamide - 500/mg/m^2/d (3d); Modified LD: Fludarabine - 30/mg/m^2/d (4d) + Cyclophosphamide - 750/mg/m^2/d (3d); Enhanced LD: Fludarabine - 30/mg/m^2/d (4d) + Cyclophosphamide - 1000/mg/m^2/d (3d).*

RESULTS

Figure 1: Effective CAR T cell dose[3] relates to CAR T kinetics, while differentiated (effector) CD4 CAR T cell dose[4] relates to potential for ≥ Grade 3 toxicity.

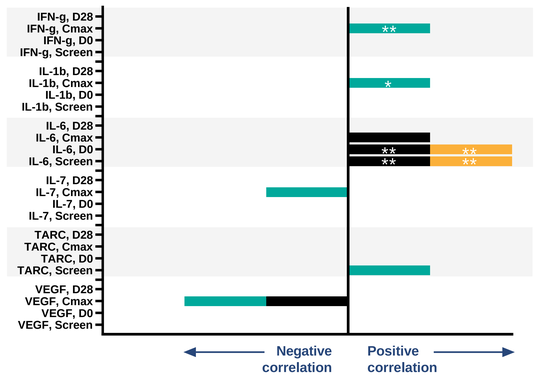


- 4:8 ratio correlates with CD4 CAR T expansion (p=0.002).
- CAR T expansion, particularly CD4 CAR T expansion is correlated with neurotoxicity in CAR T relapsed subjects that were conditioned with an intensified lymphodepletion treatment regimen.

All correlations displayed meet minimum criteria based on Pearson correlation coefficients, ρ(rho)≥0.35 and p≤0.05.
*denotes p≤0.01; ** denotes p≤0.005.

[3]Effective CAR T cell dose is equivalent to the # of non-apoptotic $CCR7^+$ CAR T cells infused.
[4]Differentiated (effector) CD4 CAR T cell dose is equivalent to the # of non-apoptotic $CCR7^-$ CD4 CAR T cells infused
[5]CAR Kinetics refer to CAR T cell expansion (AUC and/or C_{max})

Figure 2: CAR T cell expansion[6] is correlated with a recognized inflammatory cytokine profile at C_{max}.



- IFN-gamma & IL-1b correlated with CD4 CAR T expansion at C_{max} (p=0.003 & 0.009, respectively).
- IL-6 correlated with CD8 CAR T & overall CAR T cell expansion from screen (p=0.0004 & p=0.0002, respectively) to C_{max}.
- IL-7 and VEGF inversely correlated with CD4 CAR T/overall CAR T expansion at C_{max}.

All correlations displayed meet minimum criteria based on Pearson correlation coefficients, ρ(rho)≥0.35 and p≤0.05.
*denotes p≤0.01; ** denotes p≤0.005.

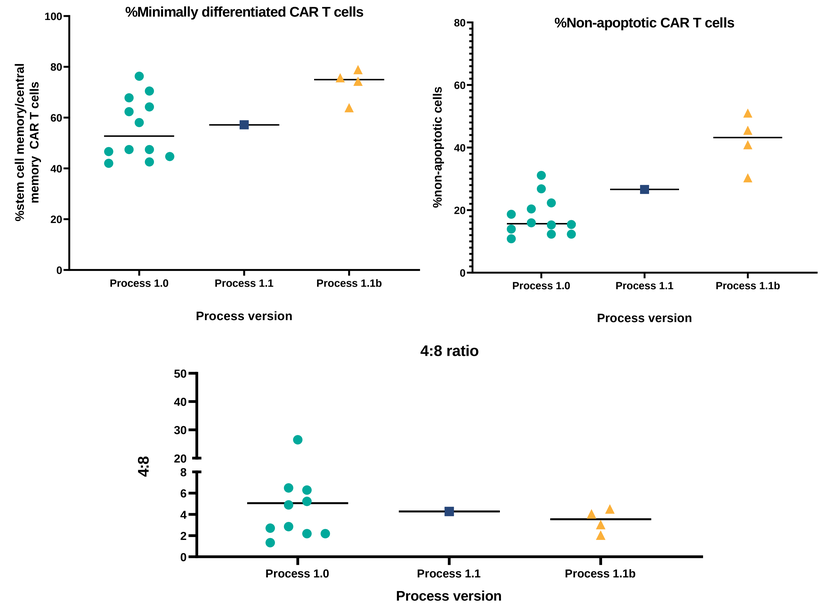
[6]CAR T cell expansion refers to AUC and/or C_{max}

Figure 3: Severe ICANS is associated with Ang2:Ang1 ratio at screen, and IL-2, IL-6, and IP-10 at C_{max}.

- Grade 3 ICANS is strongly associated with D0 IL-2 (p=0.003), Ang2:Ang1 at screen (p=0.0069), and IP-10 at C_{max}.
- Grade 2 ICANS is strongly associated with Day 0 Ang2:Ang1 (p=0.0006), IL-2Ra/CD25 (D28, p=0.0001), IL-6 (Screen, p=0.0011; C_{max}, p=0.001), IL-8 (C_{max}, p=0.0044), IL-12/IL-23p40 (D28, p=0.001), IL-15 (D0, p=0.0005; D28, p=0.0023).

All correlations displayed meet minimum criteria based on Pearson correlation coefficients, ρ(rho)≥0.35 and p≤0.05.
*denotes p≤0.01; **denotes p≤0.005.

Figure 4: Improvements to PBCAR T manufacturing increased % of CAR T attributes associated with cell viability, desired memory T cell subtype and tightened 4:8 ratio.



- Scatter dot plots display the median line for each population.
- Median, desired memory T cell subtype proportion from Process 1.0 to 1.1b increased from 52.75 to 75.00.
- Median, % healthy cells proportion from Process 1.0 to 1.1b increased from 15.67 to 43.20.
- 4:8 range decreased from 25.16 (1.34-26.5) to 2.47 (2.04-4.51).

CONCLUSIONS

1. This is the first analysis of an allogeneic CD19 CAR T product composition demonstrating relationships between post-thaw effective cell dose, pharmacokinetics, pharmacodynamics, and clinical outcome.
2. Post-thaw product composition and effective CAR T cell dose are predictive for response to treatment with Azer-cel. Peak CAR T expansion, a key determinant of durable response, strongly correlated with effective CAR T dose.
3. CD4:CD8 ratio strongly correlated with patient *in vivo* CAR T cell expansion, notably CD4 CAR T expansion. Like data reported in auto CAR T studies, CD4 CAR T expansion correlated with ≥Grade 3 neurotoxicity. This was particularly observed in a subset of patients that were both CAR T relapsed and conditioned with an intensified lymphodepletion regimen.
4. Severe toxicity is characterized by a recognized inflammatory cytokine profile, including markers of vascular activation such as Angiopoietin2 (Ang2).
5. Precision BioSciences has applied optimizations across all allogeneic CAR T platforms with the goal of improving those product attributes and characteristics that drive predictability, reliability, and performance.

Acknowledgments: The Authors would like to thank Mark Johnson, Aaron Martin, Vladimir Senyukov, James Bolling, Michael Karg, Monika Vainorius, and Chris Heery for their contributions.