Earlier use of Ciltacabtagene Autoleucel (Cilta-cel) is Associated With Better Immune Fitness and Stronger Immune Effects as Shown by Correlative Analysis of Peripheral Blood and the Bone Marrow Tumor Microenvironment (TME) From the CARTITUDE-4 Study

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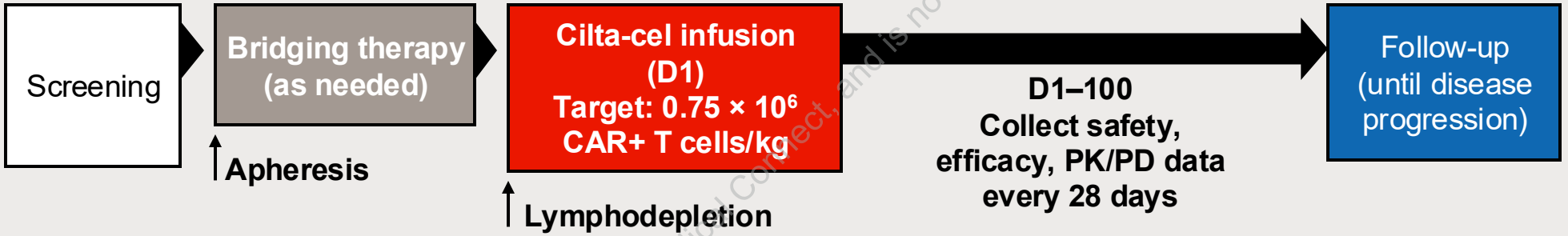
Introduction: CARTITUDE Study Designs and Hypothesis for Correlative Analysis

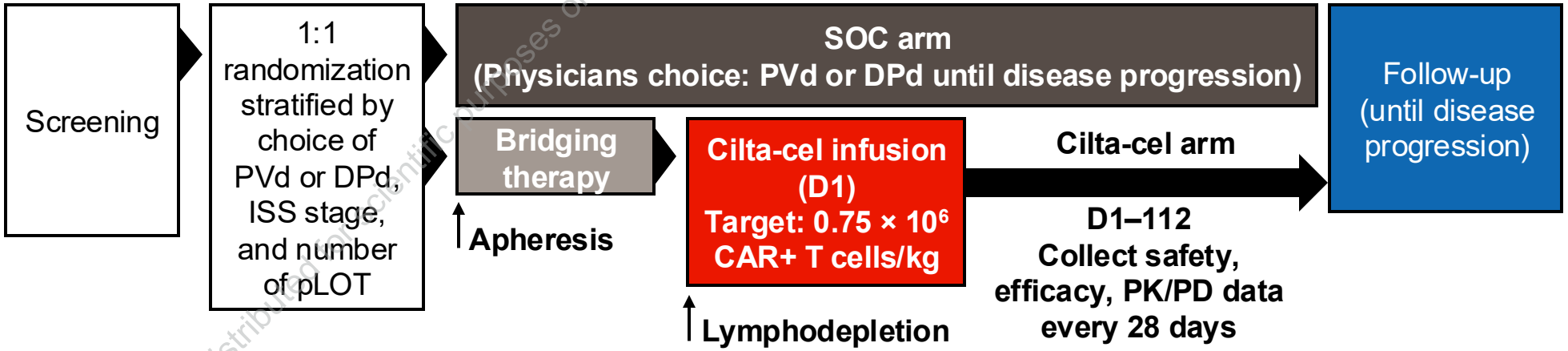
CARTITUDE-1[1]

- Phase 1b/2 study
- ≥3 pLOT or double refractory to a PI and IMiD
- Exposed to a PI, IMiD, and anti-CD38 mAb



CARTITUDE-4[2]

- Phase 3 study
- 1–3 pLOT including a PI and IMiD
- Len-refractory



CAR, chimeric antigen receptor; cilta-cel, ciltacabtagene autoleucel; DPd, daratumumab, pomalidomide, and dexamethasone; IMiD, immunomodulatory drug; ISS, International Staging System; Len, lenalidomide; PI, proteasome inhibitor; PD, pharmacodynamic; PK, pharmacokinetic, pLOT, prior line of therapy; PVd, pomalidomide, bortezomib, and dexamethasone; TME, tumor microenvironment.

1. Berdeja JG, et al. *Lancet* 2021;398:314-24. 2. San-Miguel J, et al. *N Engl J Med* 2023;389:335-47.

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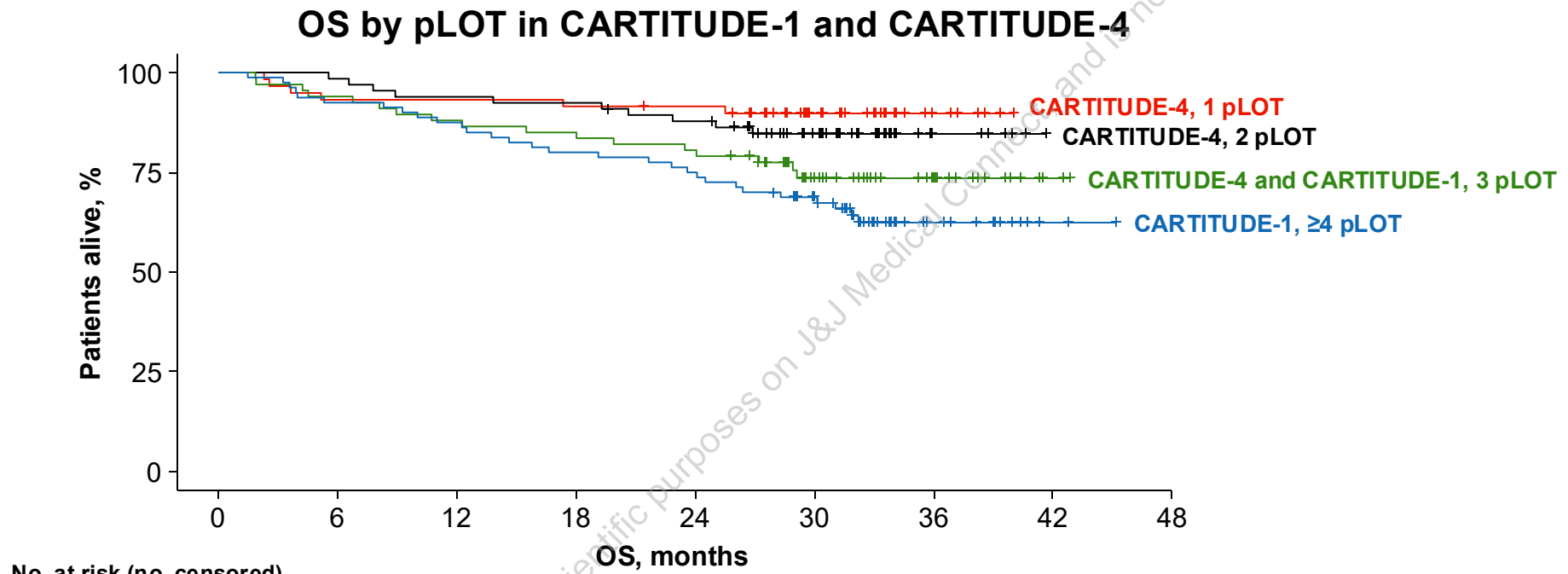
Introduction: Survival Outcomes With Cilta-cel

- Cilta-cel, a BCMA-directed CAR-T cell therapy has shown profound efficacy in patients with RRMM[1,2]
  - **CARTITUDE-1**: one-third of patients were treatment- and progression-free for ≥5 years after a single cilta-cel infusion[3]
  - **CARTITUDE-4**: significant OS benefit with a higher proportion of patients achieving deep, sustained MRD negativity with cilta-cel vs SOC[4-6]
- **PFS improves when cilta-cel is used earlier in RRMM:**

	CARTITUDE-1 TCE RRMM ≥3 pLOT	CARTITUDE-1 + CARTITUDE-4[a] TCE RRMM 3 pLOT	CARTITUDE-4[a] RRMM 1-3 pLOT
N	**97**	**34**	**176**
mPFS, months	34.9	50.4	NR (mFU[b]: 34.0 months)

[a]As-treated population. [b]mFU from randomization.
BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; cilta-cel, ciltacabtagene autoleucel; FU, follow-up; MRD, minimal residual disease; pLOT, prior line of therapy; RRMM, relapsed/refractory multiple myeloma; TCE, triple-class exposed.
1. Berdeja JG, et al. *Lancet* 2021;398:314-24. 2. Martin T, et al. *J Clin Oncol* 2023;41:1265-74. 3. Jagannath S, et al. *J Clin Oncol* 2025;43:2766-2771. 4. San-Miguel J, et al. *N Engl J Med* 2023;389:339-47.
5. Mateos M-V, et al. *Clin Lymphoma Myeloma Leuk* 2024;24(suppl 2):S290. 6. Popat R, et al. *Transplant Cell Ther* 2025; 31(suppl):S35.

Introduction: Earlier Use of Cilta-cel Results in Higher OS Rates



No. at risk (no. censored)									
1 pLOT	60 (0)	56 (0)	56 (0)	55 (0)	54 (1)	28 (26)	6 (48)	0 (54)	0 (54)
2 pLOT	66 (0)	65 (0)	62 (0)	61 (0)	57 (1)	34 (22)	6 (50)	0 (56)	0 (56)
3 pLOT	67 (0)	63 (0)	59 (0)	57 (0)	54 (0)	32 (18)	15 (35)	2 (48)	0 (50)
≥4 pLOT	80 (0)	74 (0)	70 (0)	64 (0)	60 (0)	48 (7)	12 (39)	2 (49)	0 (51)

Cilta-cel treatment in earlier lines may lead to improved outcomes due to a more immunocompetent TME

Median follow-up for CARTITUDE-1: 33.4 months. Median follow-up for CARTITUDE-4: 30.5 months from cilta-cel infusion.
Cilta-cel, ciltacabtagene autoleucel; pLOT, prior line of therapy.

CARTITUDE Studies Correlative Analysis: Methods

176 patients in CARTITUDE-4 and 97 patients in CARTITUDE-1 received cilta-cel as study treatment

Screening
- **Samples available from bone marrow**
  - CARTITUDE-4
    - n=152

Enrollment
- **Samples available from peripheral blood**
  - CARTITUDE-1
    - n=84
  - CARTITUDE-4
    - n=164

D28
- **Samples available from bone marrow**
  - CARTITUDE-4
    - n=134

6M
- **Samples available from bone marrow**
  - CARTITUDE-4
    - n=133

TME was evaluated in BMA from CARTITUDE-4 by bulk RNA sequencing

- Gene set variation analysis[1] and mixed-effects models were used to identify gene signatures and pathways modulated longitudinally and in association with PFS as well as pLOT
- TCR repertoire analysis was also performed in BMA from a subset of patients in CARTITUDE-1[2]

We evaluated TME biomarkers associated with number of pLOT to assess their impact on long-term clinical response in CARTITUDE-1 and CARTITUDE-4 studies

BMA, bone marrow aspirate; cilta-cel, ciltacabtagene autoleucel; pLOT, prior line of therapy; TCR, T cell receptor.

1. Hanzelmann S, et al. *BMC Bioinformatics* 2013;14:7. 2. Vieira dos Santos J, et al. *Blood Adv* 2025; https://doi.org/10.1182/bloodadvances.2025018078. Epub ahead of print.

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- Gene set variation analysis[2] and mixed-effects models were used to identify gene signatures and pathways modulated longitudinally and in association with PFS as well as pLOT
- TCR repertoire analysis was also performed in BMA from a subset of patients in CARTITUDE-1[1]

Immunophenotyping by flow cytometry was performed on peripheral blood samples collected from both the CARTITUDE-1 and CARTITUDE-4 studies

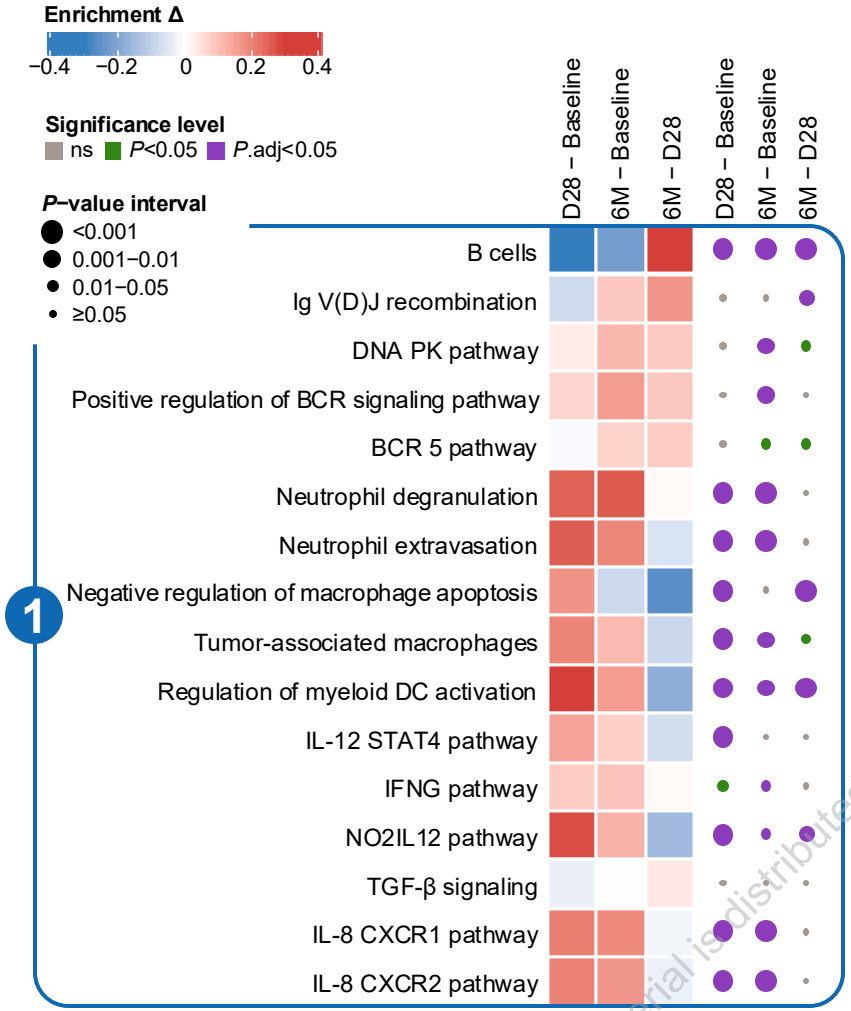
- Immune fitness at baseline was assessed in patients by pLOT and in association with PFS using nonparametric statistics

We evaluated TME biomarkers associated with number of pLOT to assess their impact on long-term clinical response in CARTITUDE-1 and CARTITUDE-4 studies

BMA, bone marrow aspirate; cilta-cel, ciltacabtagene autoleucel; pLOT, prior line of therapy; TCR, T cell receptor.

1. Vieira dos Santos J, et al. *Blood Adv* 2025; https://doi.org/10.1182/bloodadvances.2025018078. Epub ahead of print. 2. Hanzelmann S, et al. *BMC Bioinformatics* 2013;14:7.

CARTITUDE-4 TME: Increased Anti-tumor Myeloid Cell Activation and Heightened Inflammatory State Post Cilta-cel Infusion



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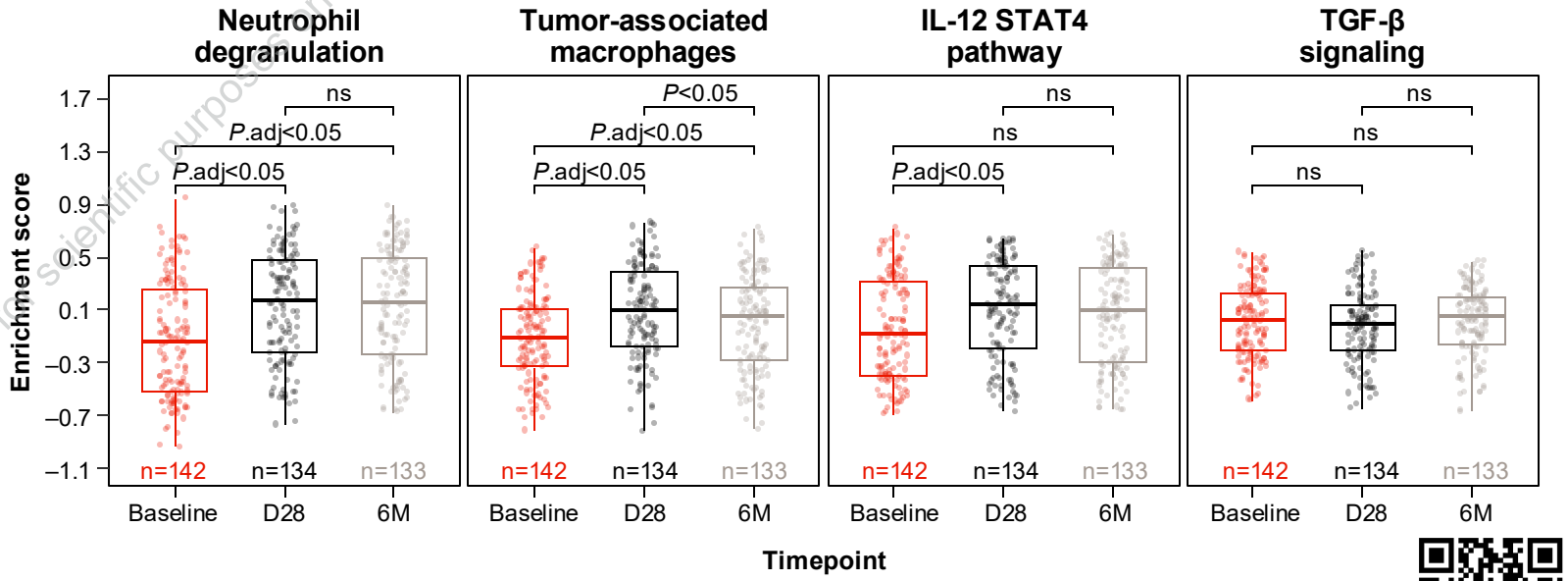
Gene expression analyses suggest:

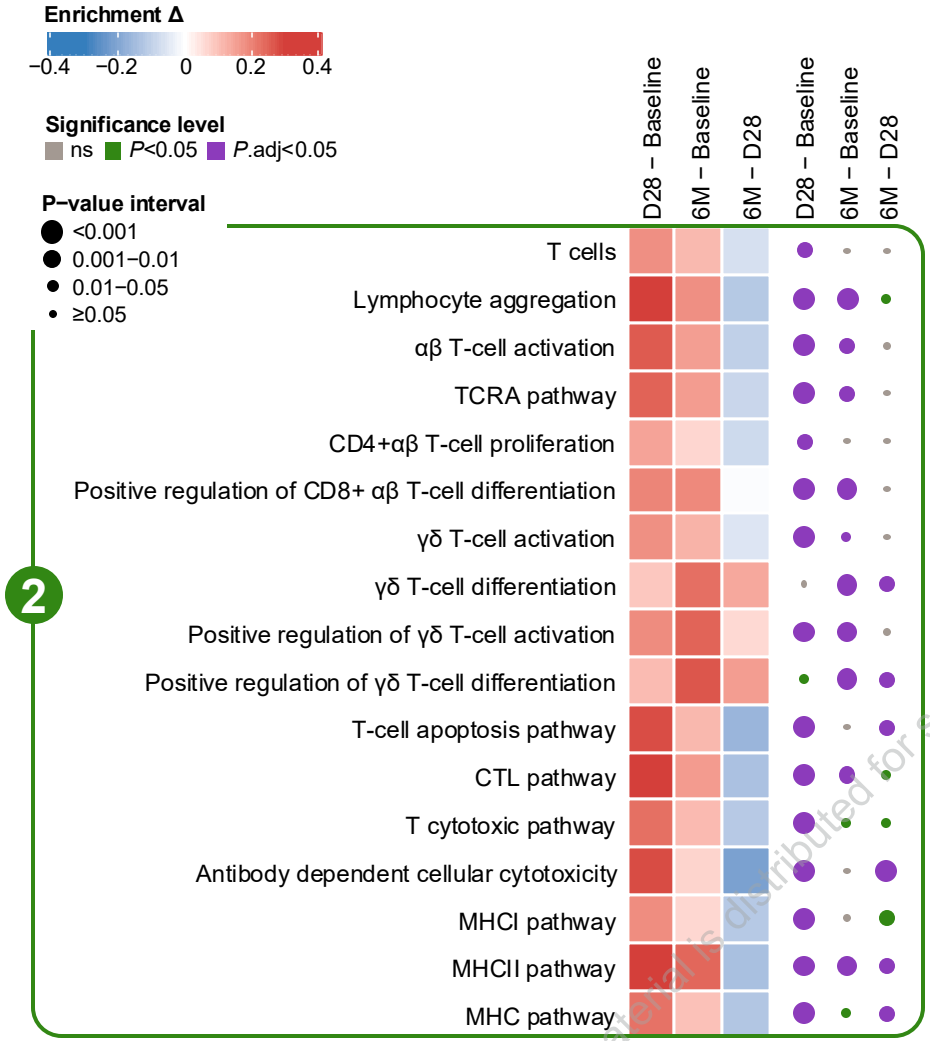
- Depleted **B cells** at D28 followed by partial recovery at 6M, accompanied by enhanced **immunoglobulin V(D)J recombination**
- Increased **neutrophil degranulation and extravasation at D28 and 6M**
- Increased **TAMs at D28 and 6M**
  - Concurrent upregulation of cytokine signaling pathways mediated by M1 macrophages (eg, IL-12), but not by M2 macrophages (eg, TGF-β) suggest TAMs are likely **M1 macrophages**[a]



[a]M1 macrophages are anti-tumor and M2 macrophages are pro-tumor.

BCR, B cell receptor; cilta-cel, ciltacabtagene autoleucel; CXCR, chemokine receptor type; DC, dendritic cell; DNA PK, deoxyribonucleic acid protein kinase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; STAT, signal transducer and activator of transcription; TAM, tumor-associated macrophage; TGF, transforming growth factor.

CARTITUDE-4 TME: Increased and Sustained T-cell Activation and Antigen Presentation Post Cilta-cel Infusion



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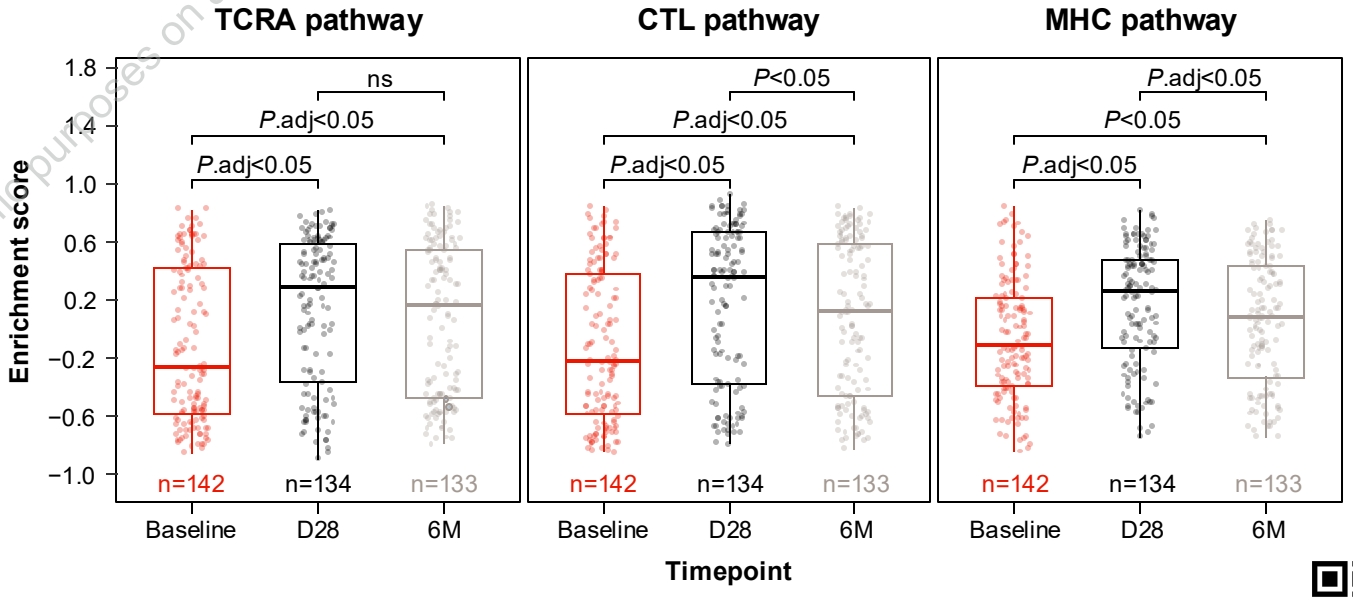
Gene expression analyses suggest:

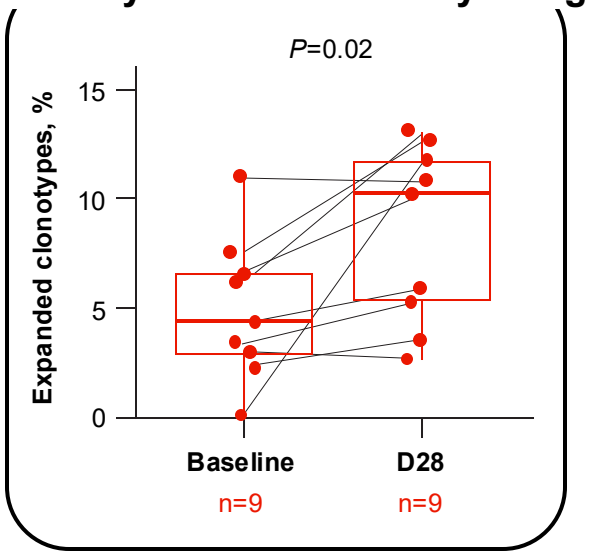
- Increased activation, differentiation, and proliferation of **diverse T-cell subtypes at D28 and 6M**
- Elevated MHCI and MHCII pathways suggesting robust **antigen presentation** capacity **at D28 and 6M**



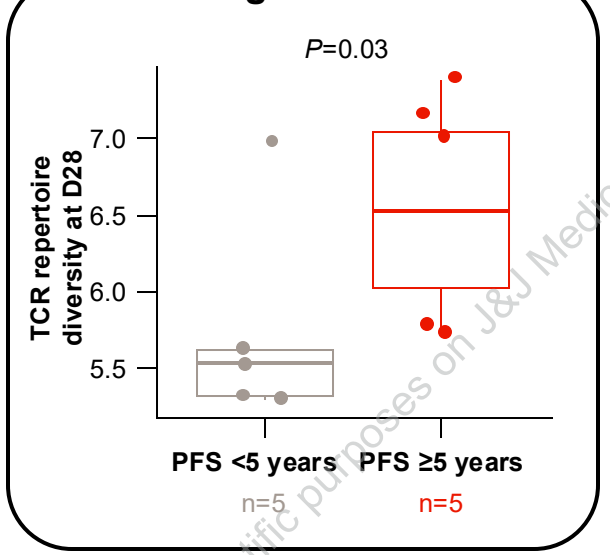
Cilta-cel, ciltacabtagene autoleucel; CTL, cytotoxic T lymphocyte; MHC, major histocompatibility complex; TCRA, T-cell receptor alpha chain.

CARTITUDE-1: Robust Endogenous Clonal Expansion of CD4 Memory T Cells and TCR Diversity Early Post Cilta-cel Infusion are Associated With Durable PFS ≥5 years[1]

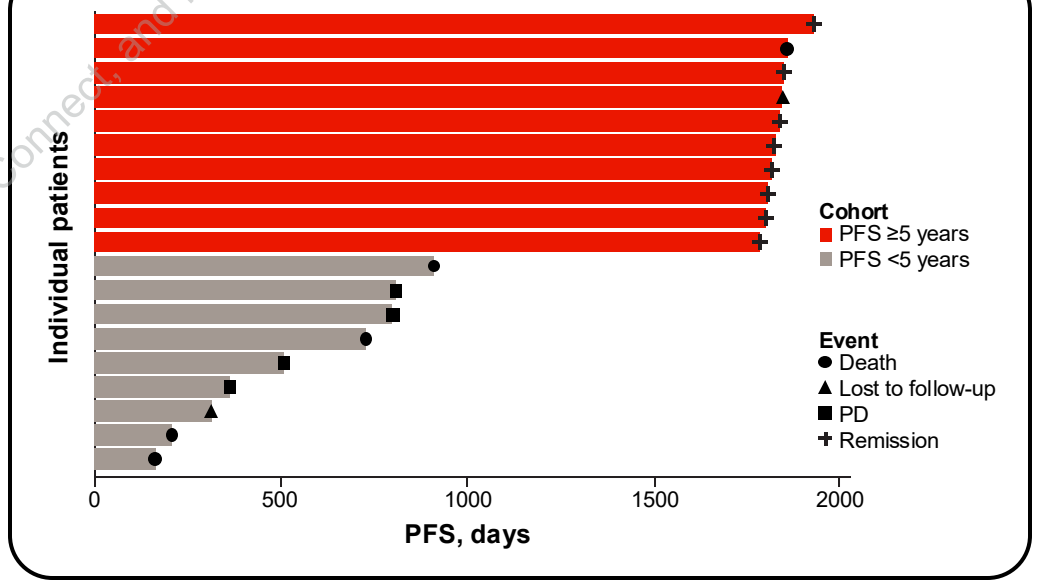
Expanded endogenous clonotypes in CD4 memory T cells in PFS ≥5 years group



TCR repertoire diversity in endogenous T cells



Swimmer plot summarizing PFS and outcomes



- TCR sequencing was performed at D28 post infusion in a subset of patients in CARTITUDE-1[a]
- Patients who achieved long-term durable responses (PFS ≥5 years) had:
  - Higher proportion of expanded clonotypes within CD4 memory T cells from baseline to D28 post infusion
  - Higher TCR repertoire diversity in endogenous T cells, which further highlights the importance of a robust TCR activation in driving long-term durable response as observed in patients with fewer pLOT in CARTITUDE-4

Enhanced TCR diversity and preferential expansion of CD4 memory T cells associated with long-term durable responses

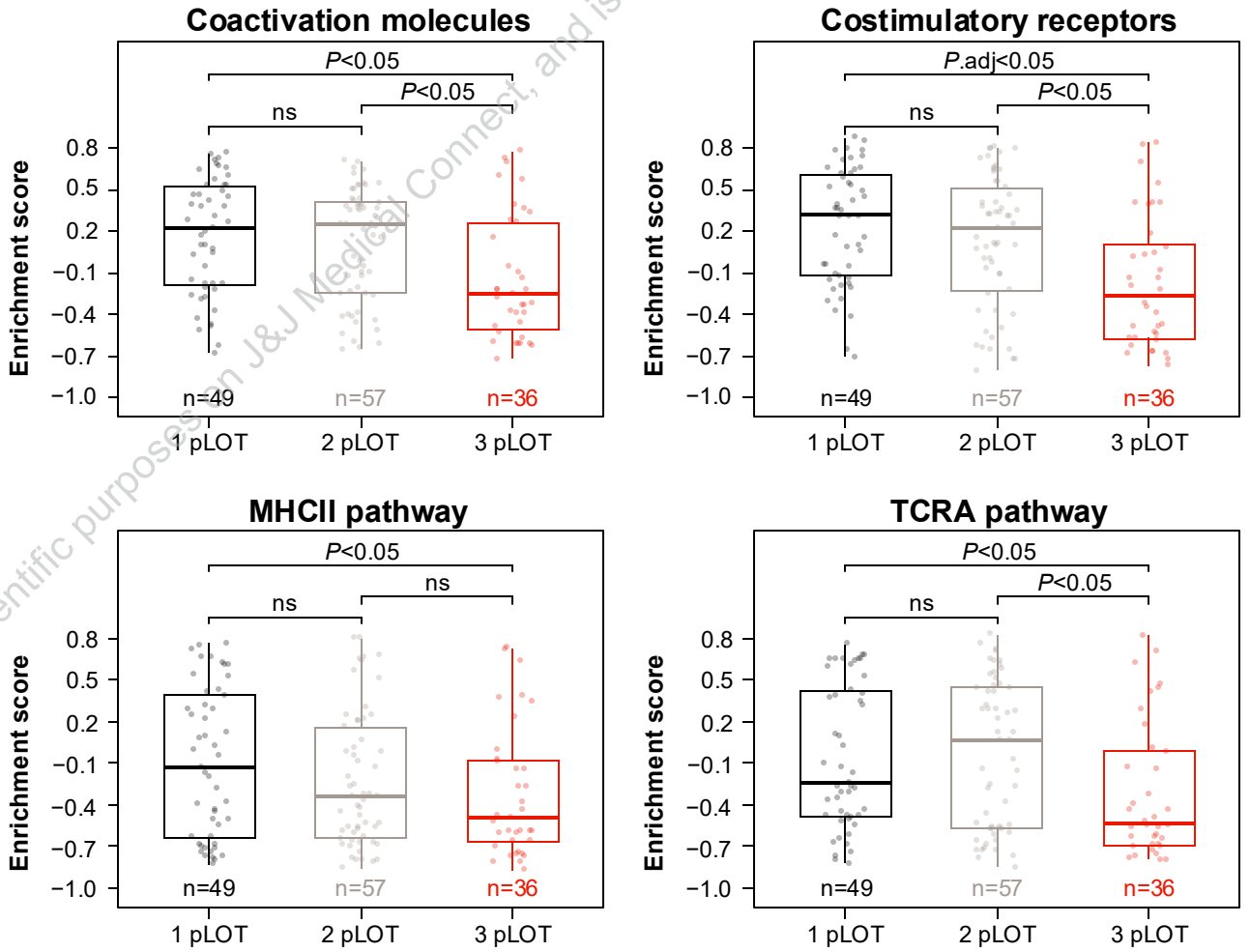
[a]treated at Mount Sinai.
cilta-cel, ciltacabtagene autoleucel; pLOT, prior line of therapy; PD, progressive disease; TCR, T-cell receptor.
1. Vieira dos Santos J, et al. *Blood Adv* 2025; https://doi.org/10.1182/bloodadvances.2025018078. Epub ahead of print.

CARTITUDE-4 TME: Patients With 1 or 2 pLOT vs 3 pLOT had a More Immunocompetent TME at Baseline

- Patients with 1 or 2 vs 3 pLOT had a more immunocompetent TME at baseline, as suggested by higher levels of costimulatory molecules, CD4+ T-cell antigen-presenting machinery (MHCII), and TCRA pathway
  - Higher levels of these gene signatures in TME at baseline trended with longer PFS (data not shown)

Earlier use of cilta-cel may support longer PFS by leveraging a more immunocompetent TME at baseline

Baseline TME gene set enrichment scores grouped by pLOT



Cilta-cel, ciltacabtagene autoleucel; MHC, major histocompatibility complex; pLOT, prior line of therapy; TCRA, T-cell receptor activation; TME, tumor microenvironment.

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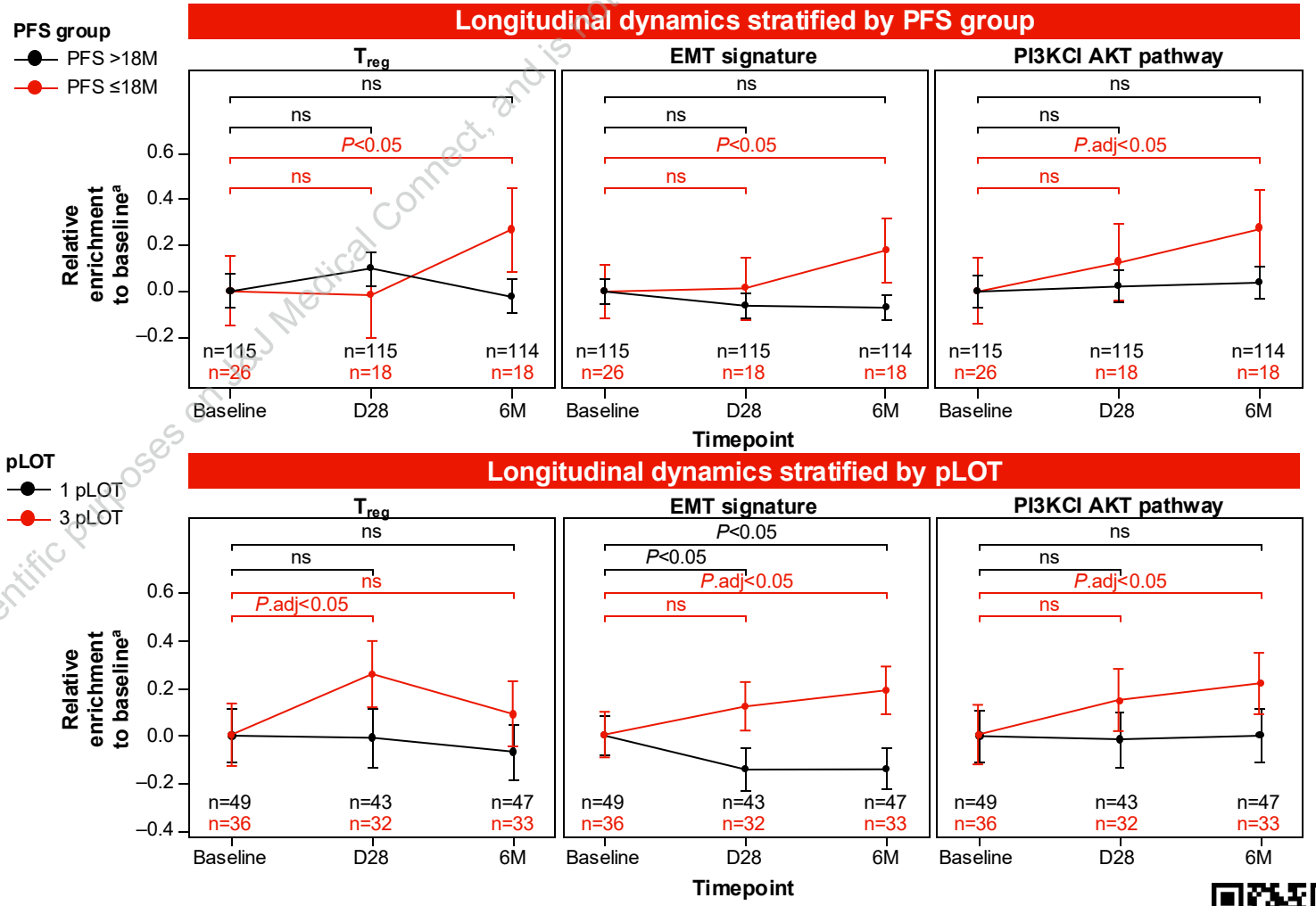
CARTITUDE-4 TME: Patients With Shorter PFS (≤18M) and Patients With More pLOT had a More Suppressive TME

Gene expression analyses suggest:

- At 6M vs baseline, patients with shorter PFS (≤18M) had:
  - Increased T_{regs}, suggestive of an increasingly more immune suppressive TME
  - Upregulated EMT signature and PI3K/AKT pathway signatures over time, suggestive of a more hostile TME[1,2]
- From baseline to D28 or 6M, upregulation of these gene signatures in the TME over time also associated with more pLOT

Earlier use of cilta-cel may support longer PFS (>18M) given a more immunocompetent TME



[a]Error bars represent estimated 95% confidence intervals.

Cilta-cel, ciltacabtagene autoleucel; EMT, epithelial-to-mesenchymal transition; PI3K, phosphoinositide 3-kinase; pLOT, prior line of therapy; TME, tumor microenvironment; T_{reg}, regulatory T cell.

1. Alameda D, et al. *Haematologica* 2020;105(9):e470-e473. 2. Heinemann L, et al. *Frontiers in Oncology* 2022;12:874325.

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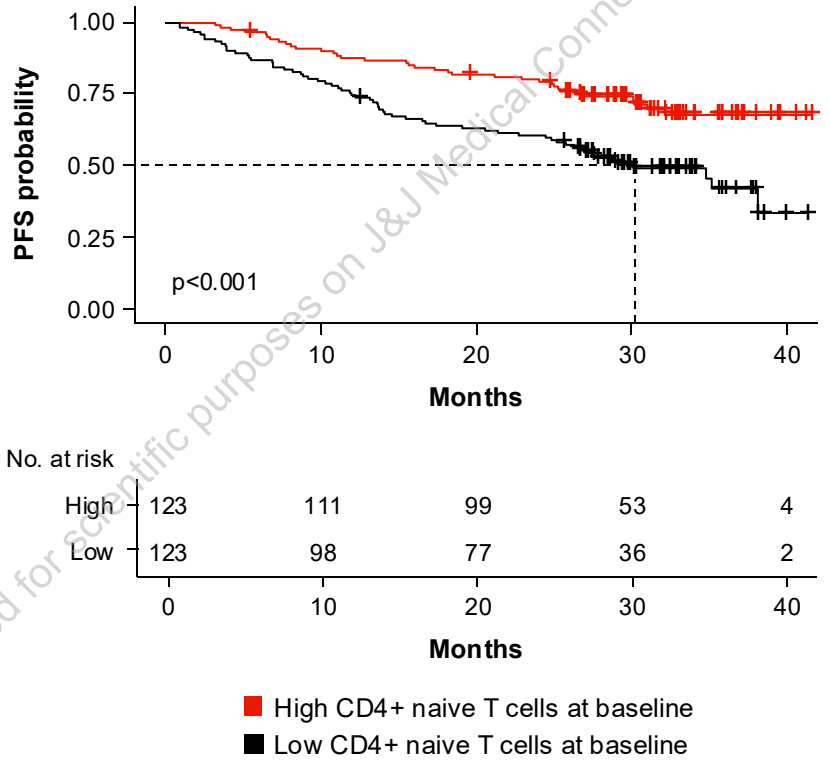
CARTITUDE-1/-4 Peripheral Blood: Patients With Longer PFS and Patients With 1 or 2 pLOT had Higher Levels of Baseline CD4+ Naïve T Cells

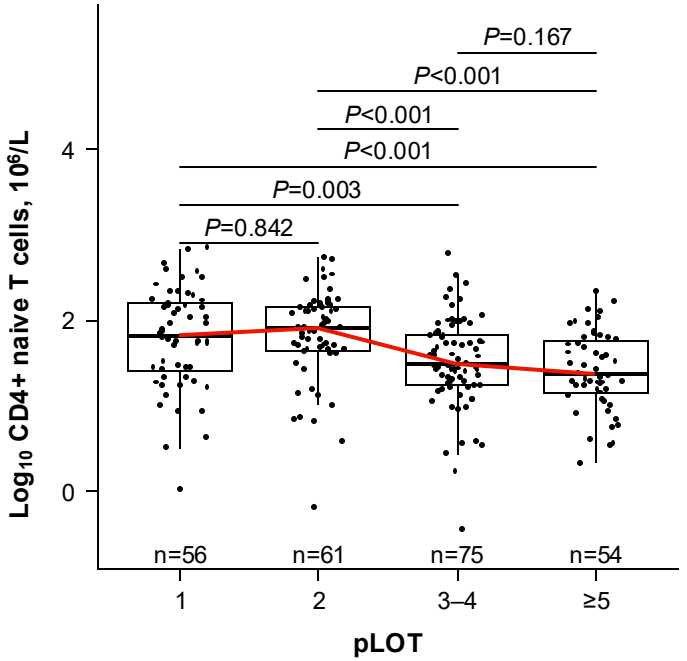
- Peripheral immune fitness at baseline was significantly enhanced in patients with fewer pLOT but not in patients with ≥3 pLOT
  - CD4+ naive T cells were previously shown to associate with long-term efficacy with other anti-BCMA CAR-T[1,2]
- Association of PFS with T-cell fitness in drug product was previously reported in CARTITUDE-1[3,4]

Better peripheral immune fitness in earlier LOT may support longer PFS with cilta-cel

PFS stratified by CD4+ naive T cells at baseline[a]



CD4+ naive T cells at baseline grouped by pLOT[a]



[a]Ad hoc analyses integrating data from CARTITUDE-1 and CARTITUDE-4 studies.
BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; cilta-cel, ciltacabtagene autoleucel; pLOT, prior line of therapy.
1. Ledergor G, et al. *Blood Advances* 2024;8:3562-75. 2. Rasche L, et al. *Blood* 2024;143(4):305-310. 3. Montes de Oca et al. American Society of Hematology (ASH) Annual Meeting 2023. 4. Jagannath et al. *J Clin Oncol* 2025 Sep;43(25):2766-2771.

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Cilta-cel in Earlier LOT: Clinical and Correlative Summary

Cilta-cel treatment in patients with earlier lines of treatment is associated with:

1. Improved patient outcomes

- Longer PFS: median PFS not reached in 1–3 pLOT vs 34.9 months in ≥3 pLOT
- Longer OS in patients receiving cilta-cel in earlier lines

2. More immunocompetent environment supporting durable CAR-T responses

- Increased expression of genes associated with anti-tumor myeloid and T cell activation, indicative of immune-engaged TME
- Increased baseline CD4+ naïve T cells in peripheral blood, indicative of immune fitness

Our data support treating patients with RRMM with cilta-cel in earlier LOT

Cilta-cel, ciltacabtagene autoleucel; RRMM, relapsed/refractory multiple myeloma; TCE, triple-class exposed; TME, tumor microenvironment.

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