Efficient T-Cell Activation and Expansion for CAR-T Therapy is Dependent on a Complex Interplay of the Starting Donor Population, Activation Antibodies and Expansion Platform

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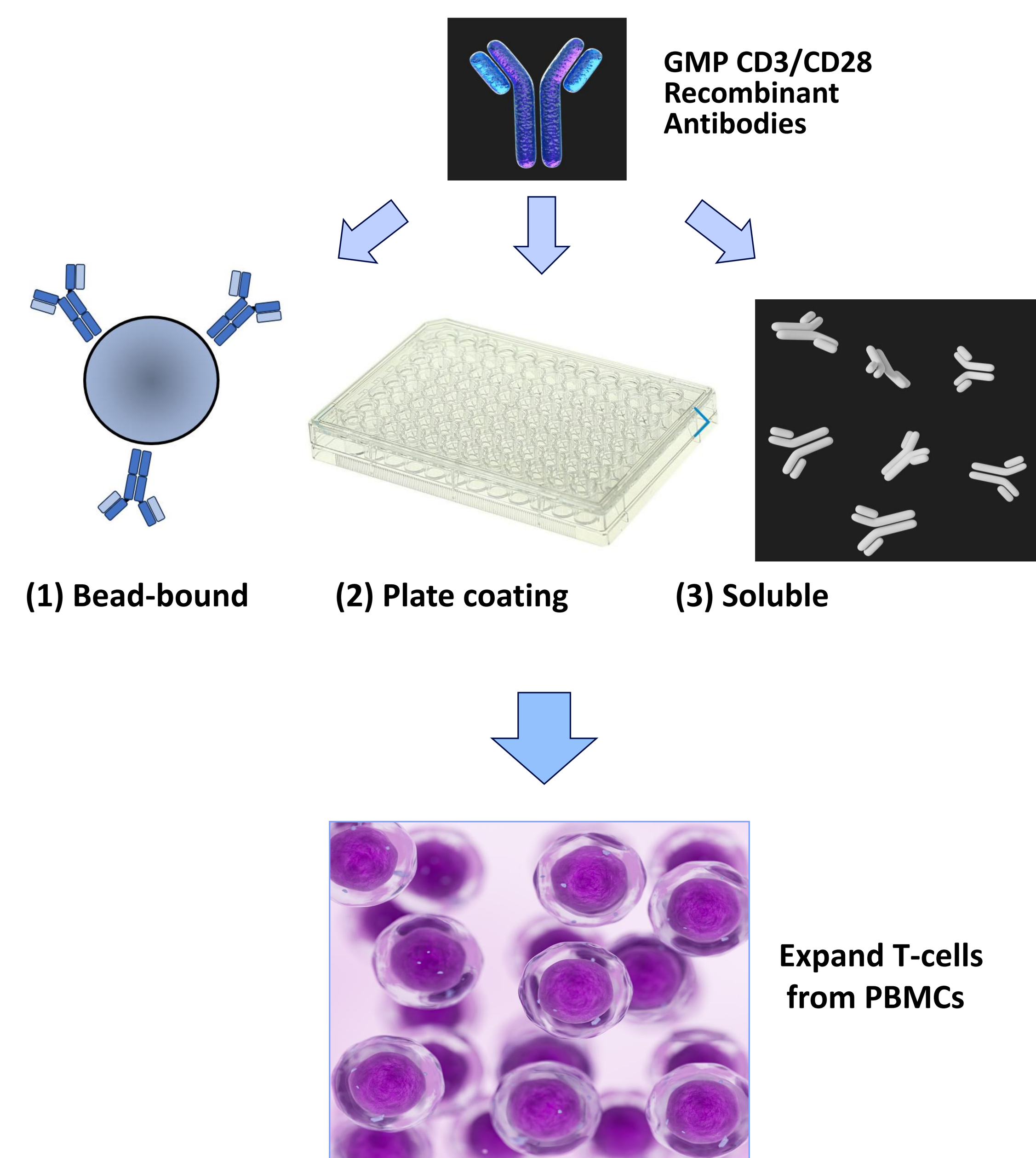
Study Design

- In the first phase of this study, T-cell activation and expansion was analyzed when CD3/CD28 antibodies were presented in 3 different modes: (1) immobilized on magnetic beads, (2) anti-CD3 coated on cell culture plates plus soluble anti-CD28 and (3) both antibodies as soluble factors.
- In a subsequent study, the effect of altering the ratio of CD3/CD28 antibodies on activation/expansion was examined.
- Lastly, the impact of the starting donor population on T-cell activation and expansion was studied, through the analysis of a larger cohort of 10 donors.
- The recombinant CD3/CD28 activating antibodies used in this study were produced under GMP conditions. This enabled a high-quality, reproducible supply of antibodies to test a variety of expansion platforms

Study Workflow



Efficient T-cell expansion can be supported by CD3/CD28 antibodies presented in different formats

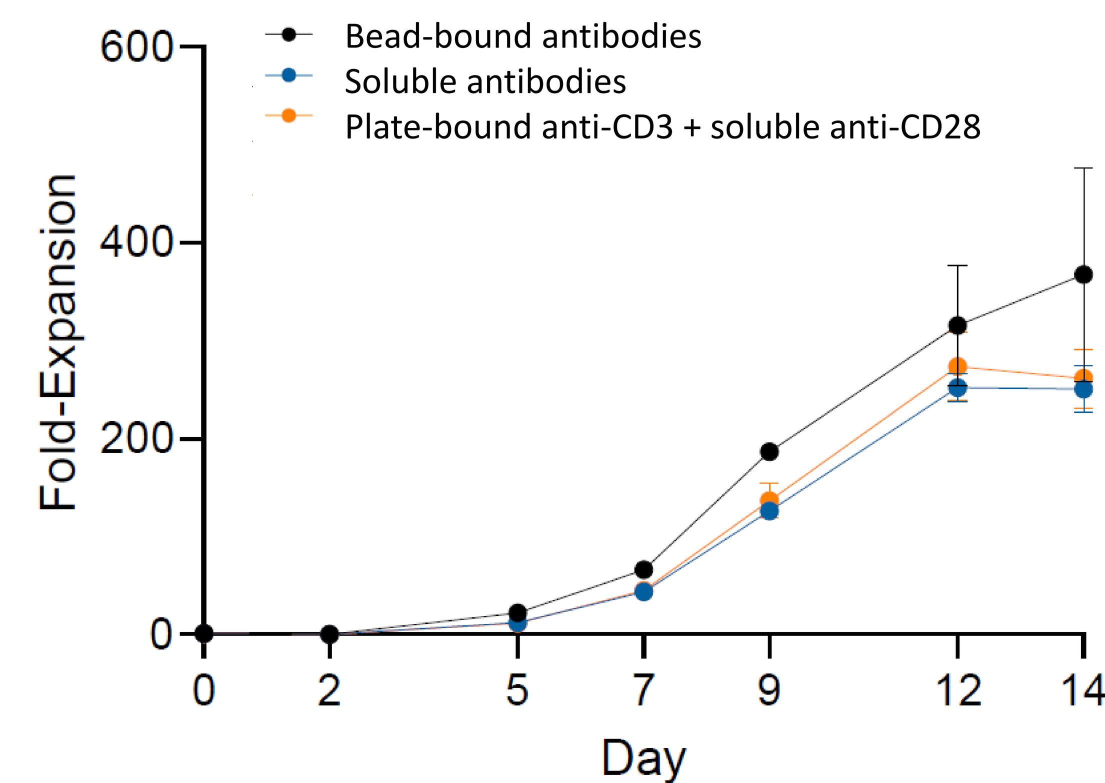


Figure 1. PBMCs from 4 different donors were used as starting material for T-cell expansions in the presence of 200 IU/mL IL-2 and CD3/CD28 antibodies. Antibodies were presented in 3 formats: (1) Bound to magnetic beads, (2) Anti-CD3 coated on cell culture plates plus soluble anti-CD28, and (3) both antibodies provided as soluble factors. Samples were harvested every 2 or 3 days to assess expansion via cell counting.

T-cell expansion mediated by bead-bound antibodies results in lower levels of the activation markers CD25 and CD69

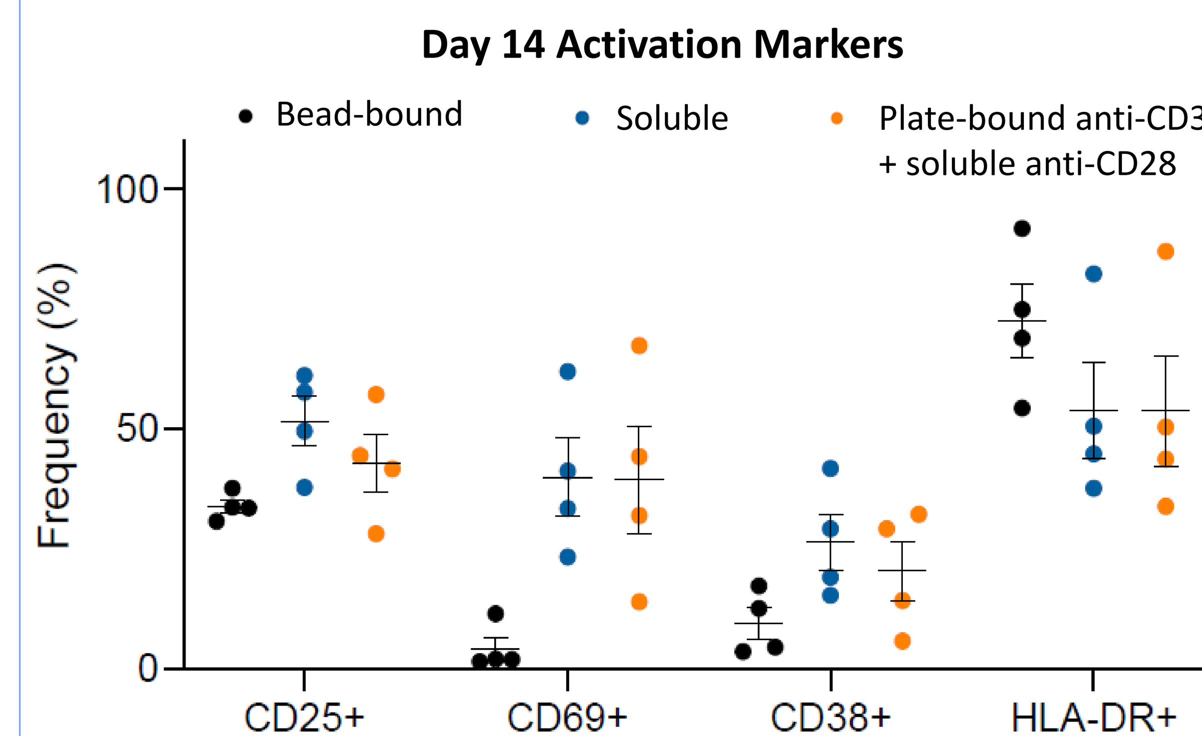


Figure 2. PBMCs from 4 different donors were used as starting material for T-cell expansions in the presence of 200 IU/mL IL-2 and CD3/CD28 antibodies. Antibodies were presented in 3 formats: (1) Bound to magnetic beads, (2) Anti-CD3 coated on cell culture plates plus soluble anti-CD28, and (3) both antibodies provided as soluble factors. Samples were analyzed by flow cytometry to assess the frequency of the activation markers on the expanded T-cell population. Activation markers at the day 14 time point are presented.

Altering CD3/CD28 antibody ratios does not impact T-cell expansion or phenotype

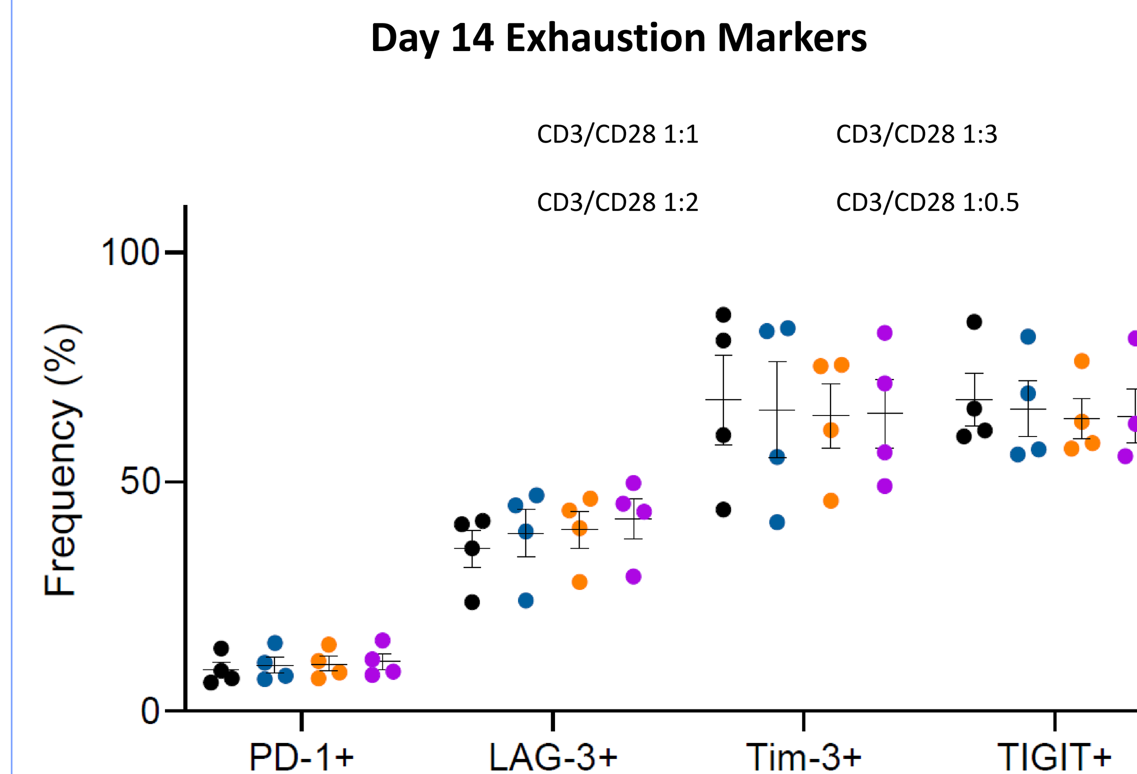


Figure 3. PBMCs from 4 different donors were used as starting material for T-cell expansions in the presence of 200 IU/mL IL-2 and different ratios of CD3/CD28 antibodies. CD3 antibodies were coated on cell culture plates while CD28 antibodies were added as soluble factors. Samples were analyzed by flow cytometry to assess the frequency of the exhaustion markers on the expanded T-cell population. Exhaustion markers at the day 14 time point are presented. T-cell expansion, activation markers (CD25, CD69, HLA-DR), and naïve/memory markers (CD45RA, CD45RO, CD62L) were also examined and did not differ among the various antibody ratios test (not shown).

The efficiency of T-cell expansion is highly dependent on the starting donor population

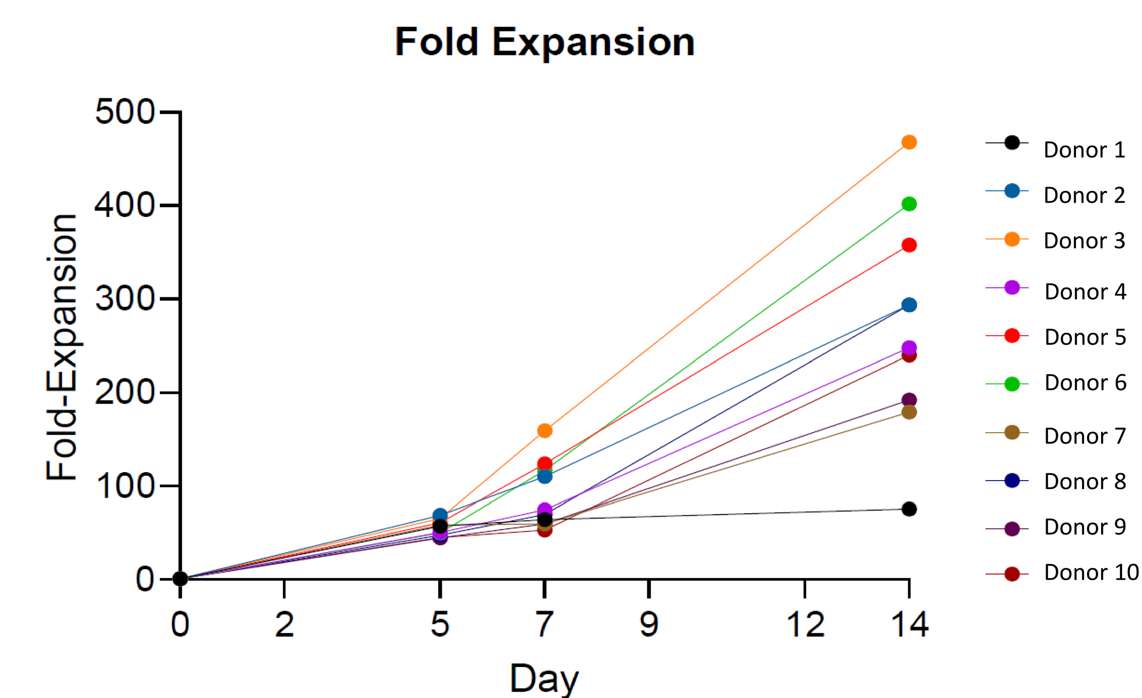


Figure 4. PBMCs from a cohort of 10 different donors were used as starting material for T-cell expansions in the presence of 200 IU/mL IL-2 and CD3/CD28 antibodies. CD3 antibodies were coated on cell culture plates and CD28 antibodies were added as soluble factors. Samples were harvested every 2 or 3 days to assess expansion via cell counting.

The phenotypic profile of T-cells expanded from PBMCs varies widely among different donors

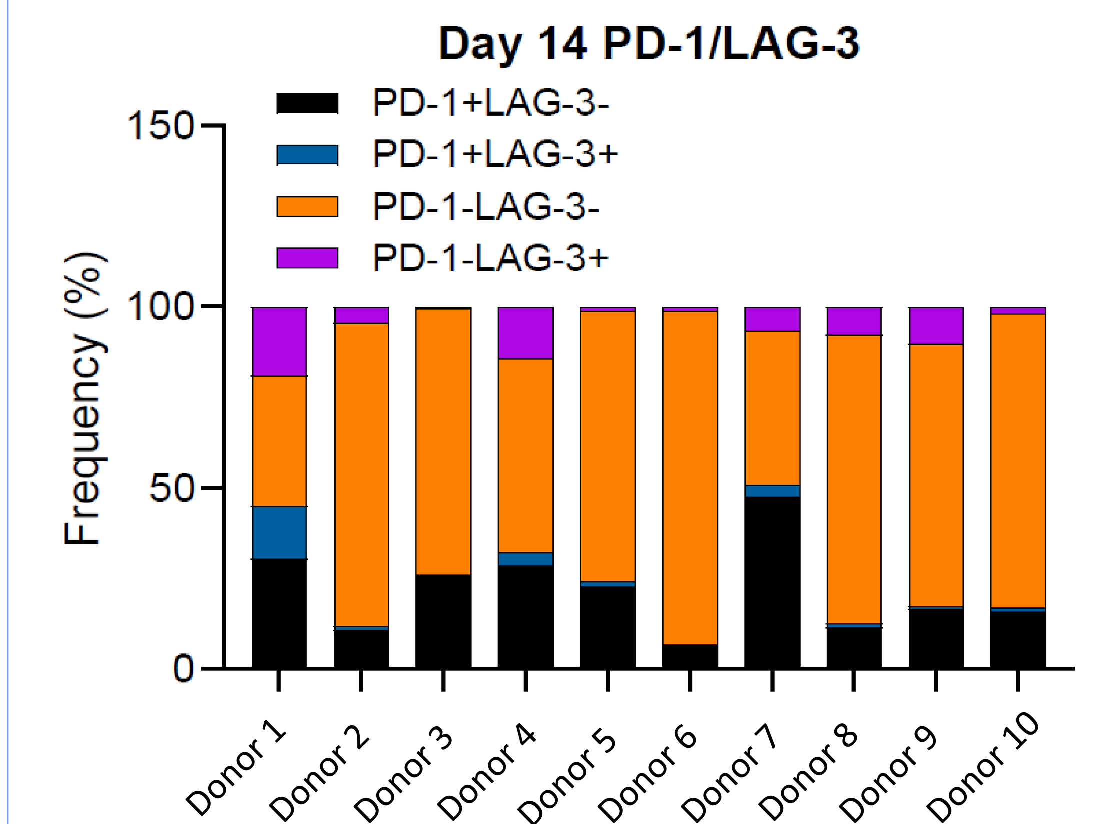


Figure 4. PBMCs from a cohort of 10 different donors were used as starting material for T-cell expansions in the presence of 200 IU/mL IL-2 and CD3/CD28 antibodies. CD3 antibodies were coated on cell culture plates and CD28 antibodies were added as soluble factors. Samples were harvested every 2 or 3 days to assess phenotype of the expanded populations. Flow cytometry data at the day 14 time point assessing cell surface exhaustion markers PD-1 and LAG-3 is presented. Activation markers (CD25, CD69, HLA-DR), exhaustion markers (Tim-3, TIGIT) and naïve/memory markers (CD45RA, CD45RO, CD62L) were also examined and displayed similar variability (not shown).

Key Takeaways:

- The extent of T-cell expansion from PBMCs was not significantly impacted when antibodies were presented in different formats (bead-bound, plate-bound, soluble), while differences were observed in phenotype.
- Surprisingly, altering the ratios of CD28 to CD3 antibodies did not modulate expansion levels or phenotypic profiles of the expanded populations.
- Significant donor-to-donor variability in expansion levels and phenotype was observed when a larger group of 10 donors was studied
- *The large donor-to-donor differences in T-cell expansion and phenotype highlight the need for a personalized approach to development of robust, reproducible manufacture of cell therapies. Rather than a static approach to T-cell activation/expansion, a tunable approach where antibodies can be tested in a variety of formats may offer better opportunities for success.*

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