Data Summary

CAR-T cells manufactured in IRO demonstrate persistent anti-tumor responses

Overview

CAR-T cell persistency, defined as the sustained ability to recognize and respond to tumor cells, is a predictor for therapeutic efficacy (Maude et al. N Eng J Med 2014; Savoldo et al. J Clin Invest 2011). In patients, especially where there is a significant tumor burden, the ability of CAR-T cells to continue responding to tumors leads to more durable anti-tumor responses and enhances therapeutic outcome.

Despite the importance of CAR-T persistency and durability, these are often overlooked or insufficiently assessed during development by many cell therapy platforms commonly used in the industry, leaving the burden on customers to assess this for themselves. At Ori, we have a biology-focused development team and are proud to extensively characterize products during IRO CAR-T protocol development, giving us and our customers confidence that IRO generates high quality functional product.

Summary

- IRO generated an average transduced cell yield of 3.4 billion CAR-T cells on day 7 compared with 1.7 billion in the control (6 donors).
- CAR-T cells manufactured in IRO demonstrated persistency as evidenced by strong anti-tumor responses in repeated challenge assays.
- CAR-T cells were able to continue to kill fresh tumor cells in a serial killing assay (comparable to control).
- CAR-T cells continued to proliferate over 35 days, with weekly tumor re-stimulation. This proliferation was significantly higher than that seen by CAR-T cells manufactured in the control.

In this application note, we present data from CAR-T cells manufactured from six donors using IRO and an industry-standard static control. CAR-T cell persistency was assessed using serial killing assay and repeat stimulation assay (measuring proliferation following tumor restimulation). We demonstrate CAR-T cells manufactured in IRO have sustained anti-tumor responses, with significantly higher antigen-dependent proliferation than those manufactured in an industry control. This data gives confidence that CAR-T cells manufactured in IRO have superior persistency and are likely to demonstrate strong and sustained anti-tumor responses in patients.

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Cell growth and CAR-T cell yield

Isolated T cells from six donors were seeded with soluble activation reagent, transduced with a commercially available CD19 CAR lentivirus and grown for a total of seven days in IRO or static control. Average viable cell yield at harvest (Day 7) was 5.3 billion in the IRO, compared with 3.6 billion cells in the control (Figure 1). Focusing specifically on transduced cell yield, a significantly higher CAR-T cell yield was obtained in IRO compared to the control (3.4 vs 1.7 billion, Figure 1). Transduction efficiency was 63.9% (54.6-72.3%, data not shown) in IRO, compared with 47.6% (39.9-56.1%, data not shown) in control.

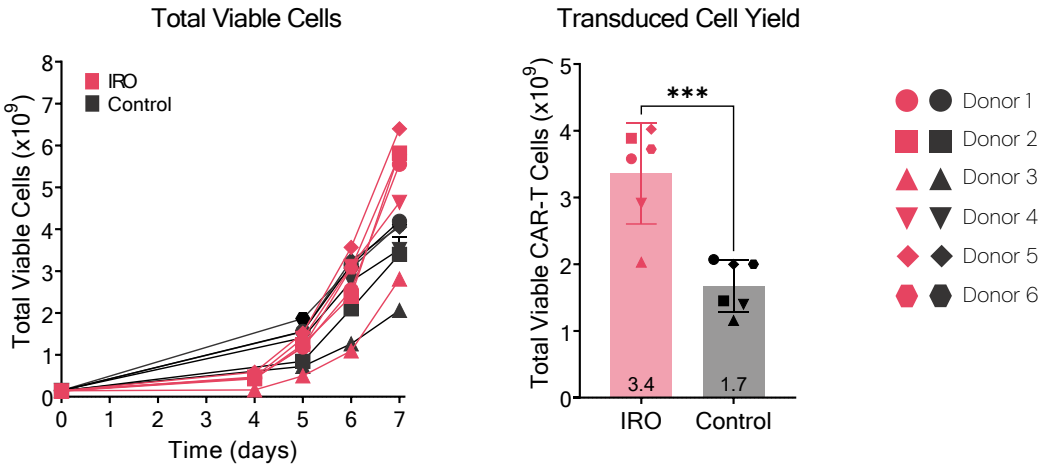

Figure 1. CAR-T cell expansion and CAR-T cell yield. Automated CAR-T cell manufacturing was run in IRO (pink), in parallel CAR-T cells were manufactured in an industry standard static control (black). Cell count and viability were assessed on Days 0, 4, 5, 6 and 7. CAR-T yield was assessed on day 7 using flow cytometry. Aggregated data is shown as mean ± standard deviation. Each donor is represented by a different symbol, as shown in the key above. CAR-T cells grown in IRO are represented by the pink symbols, while those grown in control are shown in black. Paired t test was performed to determine statistical difference, *** P<0.001.

CAR-T cells demonstrate durable anti-tumor responses

To assess CAR-T cell persistency we carried out repeat stimulation assays that measured the ability of CAR-T cells to continue responding to tumor cells over time. These assays were intended to model how CAR-T cells would respond to tumor when infused in a patient, to predict the likely durability of anti-tumor responses.

We first tested the ability of CAR-T cells to continue killing tumor cells when repeatedly given fresh tumor cells daily, in a serial killing assay. In this assay, CAR-T cells were plated with tumor cells at a fixed ratio and co-cultured in an impedance assay overnight. The following day, the CAR-T cells were harvested, counted, and re-plated with fresh tumor cells at the same fixed ratio. This process was repeated daily for four rounds of tumor stimulation, and the percentage of tumor killing was calculated at the time of harvesting CAR-T cells from the plate, as shown in Figure 2. CAR-T cells manufactured in both IRO and the industry standard control were able to continue to kill tumor cells at a comparable rate, for three rounds of stimulation, before reducing at the fourth re-stimulation.

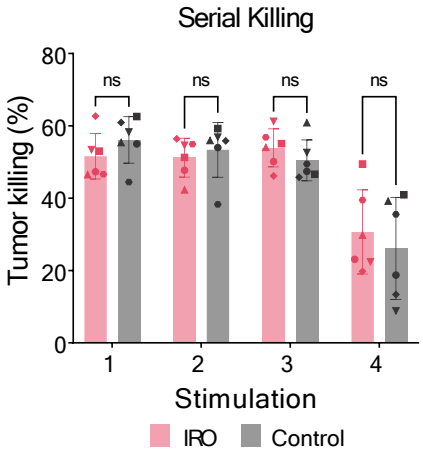

Figure 2. CAR-T cells are able to serially kill fresh tumor cells. The ability of CAR-T cells to continue to kill tumor cells was assessed using a serial killing assay. CAR-T cells manufactured in IRO (pink) or control (black) were co-cultured with a fixed ratio of tumor cells and assessed in an impedance based killing assay. Each day, CAR-T cells were harvested from the assay, counted and re-plated with a fixed ratio of fresh tumor cells. Each donor is represented by a different symbol, as shown in the key in Figure 1, above. CAR-T cells grown in IRO are represented by the pink symbols, while those grown in control are shown in black. Aggregated data is shown as mean ± standard deviation. Statistical significance was determined using 2-way ANOVA with Sidak's multiple comparisons test, ns P>0.05.

To further assess the persistency of CAR-T cells, a repeat stimulation assay measuring CAR-T cell proliferation in response to repeated tumor stimulation was carried out over a period of 5 weeks. CAR-T cells were co-cultured with tumor cells at a 1:1 ratio and, seven days later, cells were harvested and enumerated before re-plating with fresh tumor cells at the same ratio. The ability of CAR-T cells to continue proliferating was assessed by measuring the cumulative fold expansion across the course of the assay. CAR-T cells and tumor cells were quantified using flow cytometry. In addition, 24 hours after each tumor stimulation, cell culture supernatant was assessed for IFNγ, IL2 and TNFα cytokine concentrations.

As shown in Figure 3, CAR-T cells were able to proliferate for 35 days *in vitro* when re-stimulated weekly with fresh tumor cells (CAR-T cells without tumor stimulation were unable to proliferate, *data not shown*). CAR-T cells also produced IFNγ, IL-2, and TNFα at each tumor stimulation, with concentration decreasing with each repeated stimulation. CAR-T cells manufactured in IRO demonstrated superior persistency to those manufactured in the control, with a significantly higher cumulative fold increase of CAR-T cells measured following 5 tumor restimulations.

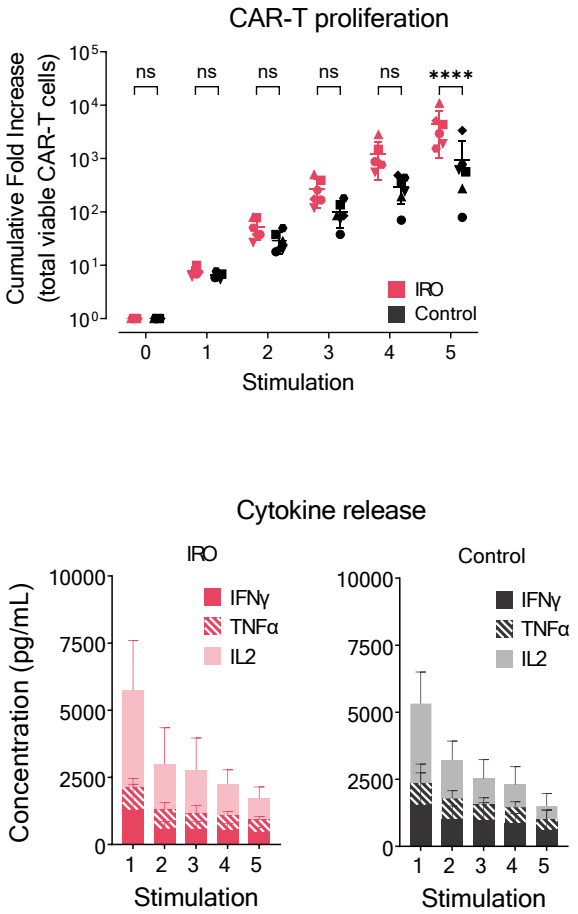

Figure 3. CAR-T cells are able to continue proliferating in response to repeated tumor stimulation. CAR-T cell persistency was assessed using a repeat tumor stimulation assay as shown in Figure 2. CAR-T cells were co-cultured with tumor cells at a 1:1 ratio and allowed to expand for 7 days. After a week, CAR-T cells were harvested, and the viable CAR-T cells per mL were measured using flow cytometry. Cells were then re-plated and stimulated with fresh tumor cells at a 1:1 ratio. Cells were re-stimulated in this way, weekly until CAR-T cells were no longer expanding. 24 hours after each tumor stimulation a small sample of cell culture supernatant was collected and assessed for cytokine concentrations. Top cumulative fold increase of CAR-T viable cells across the course of the assay. Each donor is represented by a different symbol, as shown in the key in Figure 1, above. CAR-T cells manufactured in IRO are represented with pink symbols, while those manufactured in control are represented with black symbols. Bottom the concentration of IFNγ, TNFα and IL2 was measured in the cell culture supernatant 24 hours after each stimulation. Aggregated data is shown as mean ± standard deviation. Statistical significance was determined using 2-way ANOVA with Sidak's multiple comparisons test, **** P<0.0001, ns P>0.05

Conclusions

As demonstrated in Figure 1, CAR-T cell manufacturing in IRO generates significantly higher CAR-T cell yields compared to an industry-standard static control. These CAR-T cells show sustained anti-tumor functionality (Figures 2 and 3); able to serially kill tumor, proliferate, and produce cytokines in response to repeated tumor stimulation.

This data confirms that automated manufacturing using IRO produces high-quality CAR-T cells with exceptional yield and persistent anti-tumor responses, proving that automation and superior biology can, and should, go hand in hand.

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