

A novel method for comparing the potency of CAR-NK cells to support informed donor selection

TECHNICAL NOTE

Identifying optimal donors for allogeneic cell therapies focuses on finding sustainable and potent cell sources. Until now there has not been a simple methodology to evaluate supercharged subpopulations within a heterogenous donor sample which retain a phenotype capable of serial killing. With the VivaCyte® multiparametric capability, single-cell cytotoxicity, serial killing, and phenotype can be determined on the same set of cells within a heterogeneous population.

In this technical note, we discuss the determination of the capacity of single CAR-NK cells from six individual donors to kill multiple target tumor cells. Serial killing assessment is the model used to characterize the potency of immune cells based on the number of target cells they kill over a given period. Here a serial killer is defined as a single clone that can kill more than 3 tumor target cells. The duration of each serial killing assay in VivaCyte platform is 24h with the following time points assayed: 0, 2, 6, 12, 18, 24 h. The human tumor target cell line used was Raji.

As these cells exhibit serial killing capability, it is possible to determine a novel Unified Potency Score from these data. This score calculates the weighted average number of target cells killed by a single immune cell in a standardized assay, facilitating direct comparison of donors.

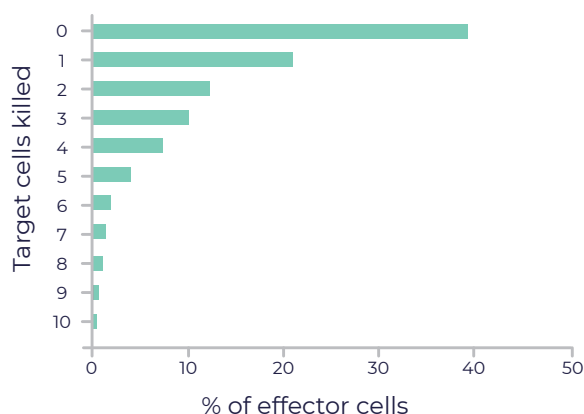
CALCULATION OF UNIFIED POTENCY SCORE

For each donor and for each effector cell population, we can define its serial killing ability as the % of effector cells (x axis) that were able to kill a certain number of target cell (y axis) in a certain amount of time, with reference to a specific target cell line. Only those microwells featuring a single effector were used, and the number of kills made normalized against the spontaneous death of target cells seen in the negative control, i.e. "no effector" microwells.

The Unified Potency Score (UPS) is the weighted average number of killed target cells by a single effector cell:

$$\text{Potency score} = \frac{1}{100} \sum_i \text{Cells killed}_i * \% \text{ of effector}_i$$

The score is calculated as the sum-product of the number of cells killed on the y-axis of the serial killing chart and the % of effector cells that made that number of kills, see Figure 1.



# cells killed	effector cells (%)
0	39.42
1	21.85
2	12.17
3	10.37
4	6.36
5	4.15
6	2.63
7	1.52
8	0.83
9	0.41
10	0.28

Figure 1: Serial killing data required to calculate the novel Potency Score, using donor B. Data was collected for a 24h period and the target cell line used was Raji.

Using the data in Figure 1 the resulting Unified Potency Score for donor B was determined as 1.63 (24h, Raji). The scores for all six donor samples were calculated and the donors ranked, Figure 2.

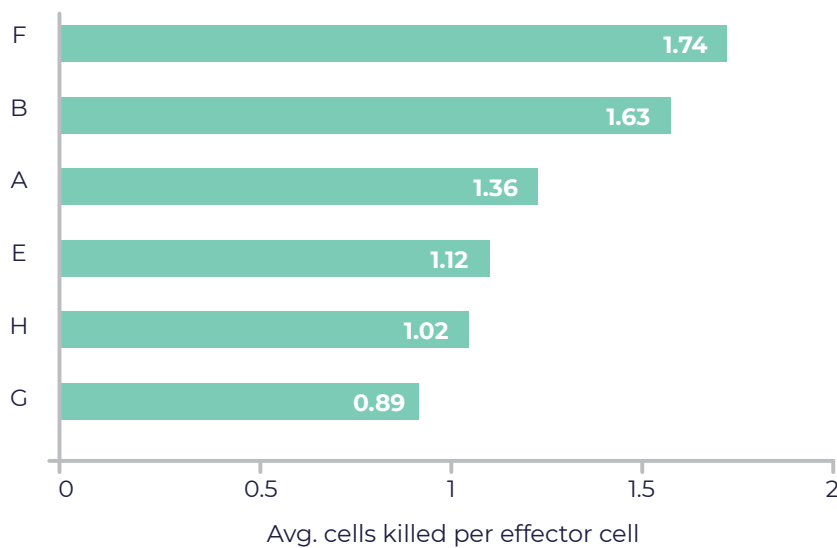


Figure 2: Ranked Unified Potency Scores, UPS (24h, Raji) for the six CAR-NK donors.

CONCLUSION

In this example six different CAR-NK donors were evaluated using the VivaCyte single-cell cytotoxicity analysis approach to enable calculation of their individual unified potency score.

Using the standard 24h, serial killing assay against human Raji target cells, the resulting UPS (24h, Raji) ranged from 1.74 down to 0.89, and the donors were ranked F, B, A, E, H, G, highest to lowest. These data have shown that, with minimal hands-on operator time, VivaCyte provides data that can comprehensively compare donors and thereby support the selection and development of CAR-NK allogeneic cell therapies with specific desired characteristics.

The UPS (24h, Raji) is a universal measure as the assay duration is set and the target cell line is a fixed standard. Using this approach, different cell therapy products, or ones produced using different processes, can now be directly compared. For example, one could compare a CAR-T product with a CAR-NK product. This novel concept of UPS (time, cell line) scores has the potential to simplify and standardize comparability across processes and product candidates.

With this information, identification of donors featuring higher serial killing activity and better killing kinetics can enable the use of a more potent cell therapy product requiring fewer immune cells to generate a response in the patient.

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