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T CELL MEMORY VARIABILITY IN HEALTHY ALLOGENEIC DONORS: RELATIONSHIP TO DONOR AGE AND EFFECT OF EX VIVO EXPANSION ON T CELL MEMORY CONTENT

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BACKGROUND

- Clinical studies have shown that durable responses using T cell therapies correlate with Tnaive/memory stem cell (Tn/scm) content, indicating that these characteristics are important for better clinical outcomes in cancer patients (Fig. 1).
- To identify optimal healthy donor starting material for allogeneic T cell therapies, Tn/scm content is one of the factors that should be assessed for T cell-based therapy donor selection.
- To address this need, Excellos, a full service CDMO, has developed a donor characterization ***Escore*** platform that assesses effector potential, metabolic fitness and T cell memory potential of healthy allogeneic T cell donors.
- The memory potential analysis is a proteomics/FACs-based method characterizing Tn/scm content and we evaluated 100 donors ranging in age from 18-78 years.

MATERIALS AND METHODS

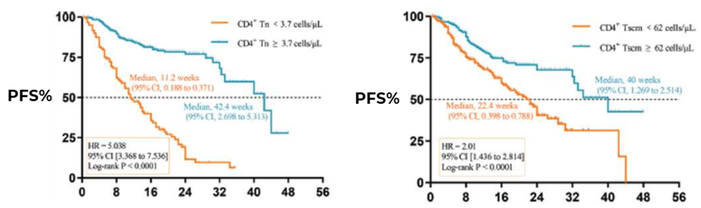
- One hundred healthy donor leukopaks were consented and collected for ***Escore*** donor characterization and memory assessments.
- Donors ranged in age from 18-78 years.
- Ficoll-enriched PBMCs isolated from the leukopaks were then used to isolate PAN CD3 T cells by negative selection using magnetic beads (Miltenyi).
- Effector potential and metabolic fitness was assessed by secretome and kinetic analysis.
- Tcell memory potential analysis using a proteomics/FACs-based method was carried out to characterize the Tnaive Tstem cell memory (Tn/scm) content.
- 14 day ex vivo expansion of the isolated T cells in T cell expansion medium containing IL2 was carried out with sample cell counts and Tn/scm analyses carried out on days 0, 7 and 14.

RESULTS

- The overall ***Escore*** varied across all donor age groups (Fig. 2).
- Low ***Escore*** donor cells are less potent in cell killing assays than high ***Escore*** donor cells. (Fig. 3).
- There was a decrease in Tn/scm content with increased age and variability in Tn/scm content for all age ranges (Fig. 4).
- Ex vivo expansion of T cells showed significant decreases in Tn/scm content on expansion days 7 and 14 (Fig.5).

FIGURE 1. T cell Naïve/Memory (Tn/scm) Clinical response correlates noted

- CD4/8 T Stem Cell Memory Cells (Tscm) and CD4/8 T Naïve Cells (Tn)
- In vivo persistence, improved PFS and increased survival correlates noted



(Zhang et al. DOI 10.3389/fimmu.2022.996348)

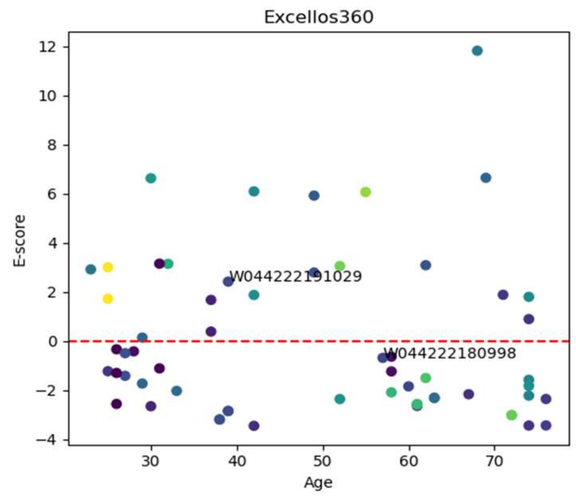
To assure more durable clinical responses therapies with greater Tscm and Tn content are needed

FIGURE 2. Figure 2. *Escore* Donor characterization

A Proprietary ***Escore*** Algorithm has been developed
Multiple weighted donor factors are incorporated into the Escore platform

- Donor demographics
- Effector potential
- Metabolic fitness

High Escore and low ***Escore*** donors can be identified



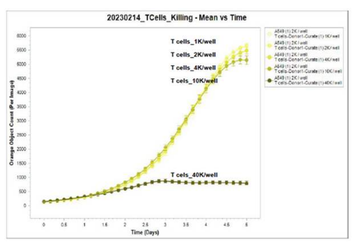
Donor ***Escore*** >0: Acceptable
Donor ***Escore*** ≤0: Unacceptable

Enables identification of preferred donors for starting material
Variable within healthy young donor cohorts

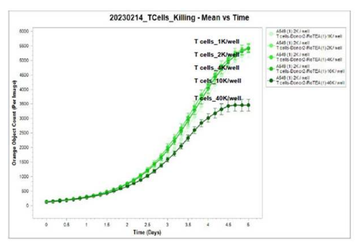
FIGURE 3. Correlation of *E360 Score* With CD3 T Cell In Vitro Killing

Results: Pan T Killing Assay:

Hi ***Escore*** Donor



Low ***Escore*** Donor



A549 cells stably transduced by Nuclight Red were seeded at 2k/well on Day 0. On Day 1, PBMCs from 2 individual donors were recovered followed by T cells isolation using Pan T cell isolation kit (Miltenyi Biotech). The purified Pan T cells were resuspended in the medium containing antibodies against CD3 and CD28, at the corresponding density, followed by addition into the 96-well plate according to the Plate Map. A549-Nuclight Red cell growth was monitored using Incucyte for 5 days.

FIGURE 4. Tn/scm Content is Variable in Donors

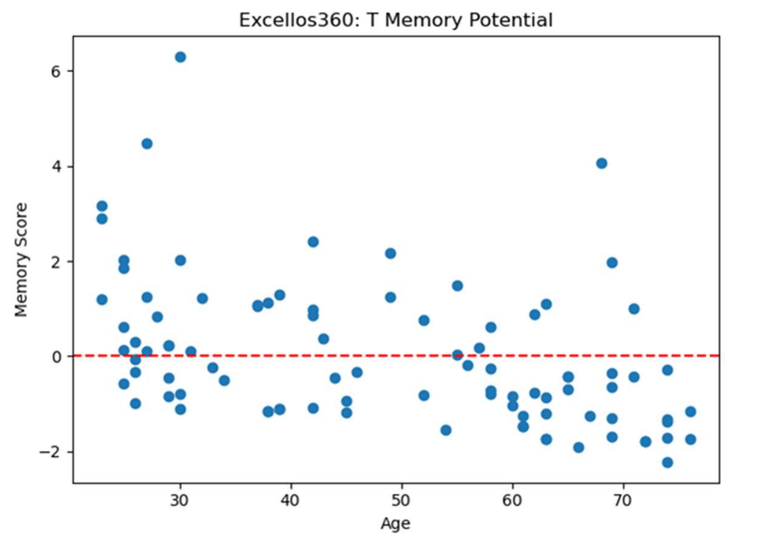
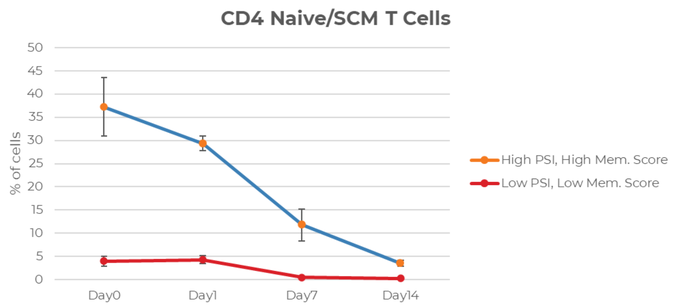


FIGURE 5. Effect of cell expansion on Tn/scm Memory Potential

CD8 Tscm/Tn assessment vs incubation time



Hi ***Escore*** donors have higher Tscm/Tn T cells
Decreased Tscm/Tn content with prolonged expansion noted

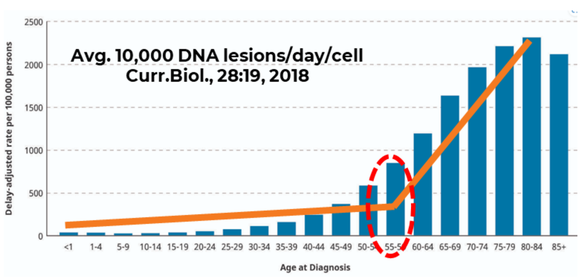
FIGURE 6. Cancer Incidence and Tn/scm Memory Correlate

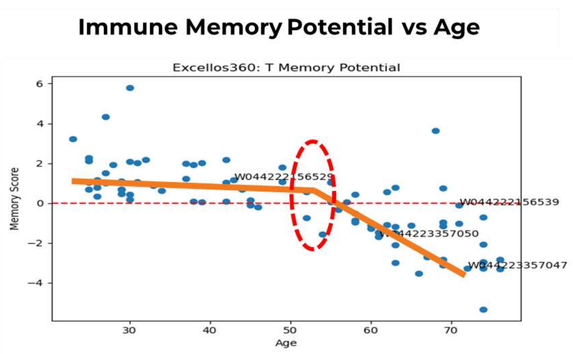
We “cure” ourselves of cancer - at least for a period of time...

But: As we age this protection is lost

Why is that?

- Not solely due to carcinogens or mutations – but please don’t smoke!
- Our immune system breaks down as it ages.





DISCUSSION

- Young donors typically considered acceptable for cell therapy starting material can have variable degrees of Tn/scm content.
- It is important to consider the Tn/scm content of donor starting material to assure better chance of durable clinical responses.
- Carrying out donor screening to assess this should be considered for optimal product properties.
- T cell expansion can have a significant impact on the Tn/scm content of the expanded product and should be monitored.
- There is decrease in Tn/scm content with increased age that is non-linear, with an inflection point in the age range of 55-65 years.
- This inflection point is coincident with an inflection point for cancer incidence in humans, potentially implicating T cell public neoantigen memory as part of the host immunoprotective system that deteriorates with age (Fig. 6).

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