CELL & GENE THERAPY INSIGHTS

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TROUBLESHOOTING SUPPLY, MANAGEMENT & OPTIMIZATION ISSUES

SPOTLIGHT

INNOVATOR INSIGHT

Improving the quality cell yield of T-cell immunotherapies through selective pressures imparted by culture media supplements

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New T-cell based therapies use the adaptive immune system as a modality in multiple blood cancer indications and are being investigated in some solid tumor indications. This study looks at both total yield and memory character as measures of cell quality and those traits were used to evaluate human AB serum (ABS) and human platelet lysate (nLiven) as culture media supplements. Two independent labs showed statistically significant increases in both total cell yield and final T-cell central memory phenotype after expanding isolated cells in medium supplemented with nLiven as opposed to ABS. There was an additional, unexpected observation of increased donor to donor consistency when cultured with nLiven which may be a result of a more homogenous source of proteins and chemicals typically required to expand T-cells. Developing commercially viable manufacturing processes for T-cell-based therapies requires the adoption of new technologies that will facilitate process robustness. This study investigates media supplements within this context.

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Engineered T-cell therapies, CAR-T and TCR, have emerged as a highly effective new therapeutic modality in blood cancers and are showing promise in solid tumor indications in the clinic. These treatments work by co-opting the immune system's natural cancer fighting ability and targeting a surface antigen specific to the cancerous cell population. Utilizing a complicated biological system provides therapy developers with a powerful tool to direct against various diseases, however it also limits researchers' understanding of the attributes that indicate the drugs' efficacy. Historically, treatment doses are calculated based on the total number of cells presenting the surface antigen of interest with the hope that a portion of those cells would engraft in the patient and exhibit a persistent response. Recent research, however, has indicated that T cells that present a central memory phenotype (TCM) have increased efficacy over effector phenotypes across multiple disease models [1–4].

Using cell memory character as a lever to increase therapeutic efficacy could have significant downstream effects for manufacturers and patients. If the treatment is more effective on a per cell basis, patients could be treated with a smaller minimum therapeutic dose. Manufacturers could have shorter manufacturing lengths, fewer materials, and increased process consistency, while patients could see a cheaper therapy that has a more consistent efficacy.

Another concern raised by therapeutics developers who have received or are anticipating market approval is the sustainability of their chosen media supplements. Historically, human primary cell culture has been limited to a small group of investigators, but recently the space has grown rapidly. That growth has put stress on the established supply chain. Fetal bovine serum has significant regulatory concerns as an animal derived reagent, so researchers have leaned heavily on human AB serum as a source of a particular mix of proteins, hormones, and cytokines that promotes growth in T-cell manufacturing processes. However, members of the industry are acutely concerned about the long-term availability of human AB serum which has to be sourced from voluntary, male donors of the AB serotype. The source of this material is relatively inflexible as only approximately 3% of the total population have the AB serotype, it cannot be scaled to meet demand like other reagents, and AB serum is used in the culture medium of most T-cell therapies [5]. The need for an alternative xeno-free supplement is rapidly approaching.

To investigate methods for improving cell quality and shore up supply concerns, a group at the Baylor College of Medicine, led by Norihiro Watanabe, performed a small-scale evaluation of the impact of various protein sources in culture media on T-cell memory phenotype and therapeutic efficacy using fetal bovine serum (FBS), human AB serum (ABS), and a uniquely processed human platelet lysate (nLiven PR™). Their results show a statistically significant increase in T cells exhibiting central memory phenotypes and in total survival using a mouse model when cultured in nLiven PR™ versus ABS and FBS [6]. Dr Watanabe's team evaluated the *in vivo* efficacy of T-cell cultured in nLiven PR™ against both solid tumors and blood cancers. The study shows that the T cells expanded *ex vivo* with nLiven PR™ had statistically significant increases in the duration that cells were present in peripheral blood, total cells that were actively circulating, and the percent survival of the mice when compared to the same population of T cells expanded with either FBS or ABS. To further evaluate the impact of each medium supplement for therapeutic effect, the study evaluates engraftment by rechallenging the solid tumor model 21 days after the initial infusion. Again, the response by the cells expanded with nLiven PR™ was significantly more pronounced than in the cell populations expanded in either FBS or ABS.

Hitachi Advanced Therapeutics Solutions (HCATS) and Sexton Biotechnologies partnered to investigate if the human platelet lysate supplement nLiven PR™ would maintain these strong advantages when evaluated in more clinically representative culture models.

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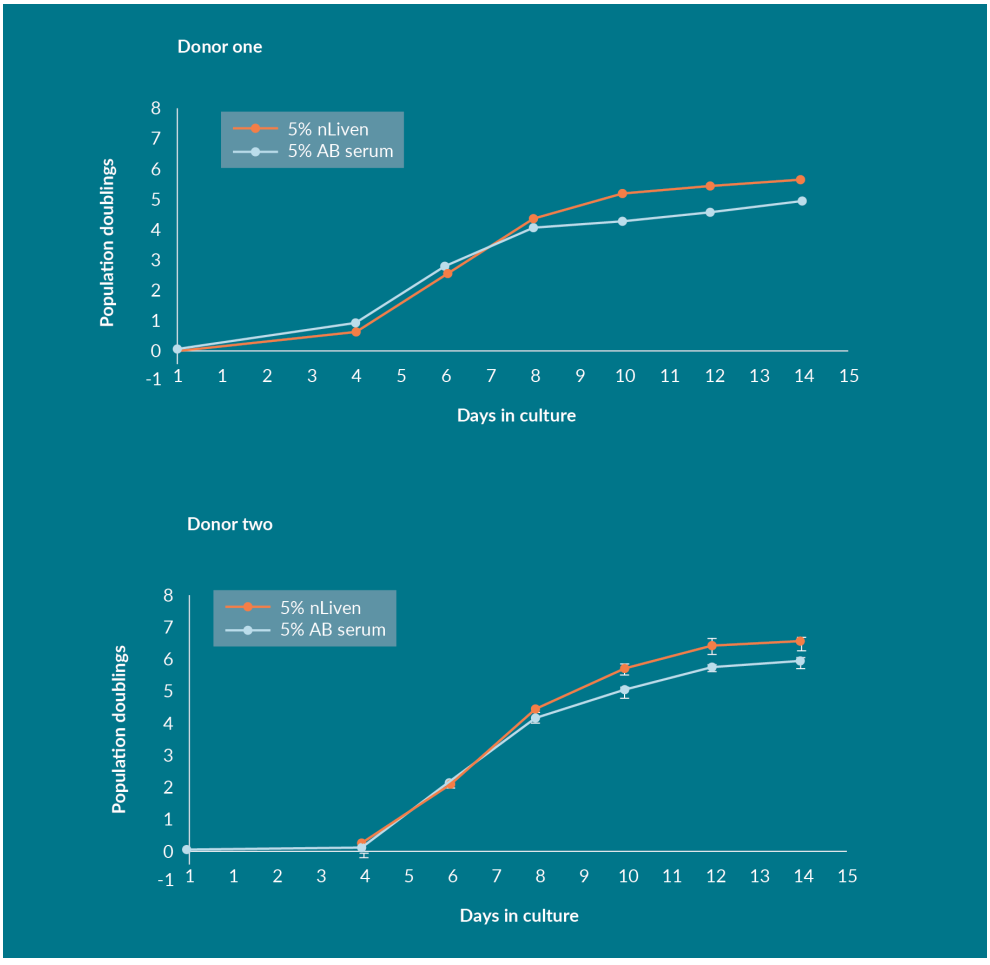
The first consideration for the experiments performed at Sexton and HCATS was to confirm that medium supplemented with nLiven PR™ produced a similar yield of total T cells to that of standard medium supplemented with ABS. At Sexton, peripheral blood mononuclear cells (PBMCs) were obtained from two donors (StemCell Technologies), activated with ImmunoCult™ Human CD3/CD28 T Cell Activator (StemCell Technologies), and cultured for fourteen days in static conditions. There was no significant change in cell expansion through the first 8 days of culture, however after day 10 the cultures using nLiven PR™ showed significantly higher expansion compared to ABS in both donors (Figure 1). Part of the increase in total expansion may be the result of a promotion of T-cell proliferation over other cell populations. This is based on a FACS analysis performed on day 8 where nLiven PR™ had increased the $CD3^+$ population to 98.2% ± 0.24% and 97.6± 0.26% for donor 1 and 2, respectively, while the ABS conditions were at 94.8% ± 0.63% and 91.9% ± 1.47% (data not shown).

Negatively selected, homogenous $CD3^+$ starting populations were obtained from three donors, activated with Dynabeads® (Thermofisher), and expanded for 11 days in stirred-tank bioreactors for the work performed at HCATS. This served to assess if the impact of using nLiven in place of standard protein supplements would persist in an

FIGURE 1

PBMCs from two donors were expanded in the labs at Sexton Biotechnologies.



In both instances, statistically significant expansion was achieved after ten days in culture (p=0.002 and p=0.009 respectively by T-test). (N=3).

agitated culture system as opposed to a static one. The average population doubling across all three donors was 6.1 ± 0.58 and 6.0 ± 1.37 for the nLiven PR™ and ABS conditions respectively (Figure 2). There was no statistically significant change to the expansion of T cells when using nLiven PR™ in the experiments performed at HCATS. Despite a similar cell expansion, the nLiven PR™ media demonstrated a coefficient of variance of 10% compared to 23% for ABS media highlighting a major decrease in donor to donor variability with the nLiven PR™ cultures.

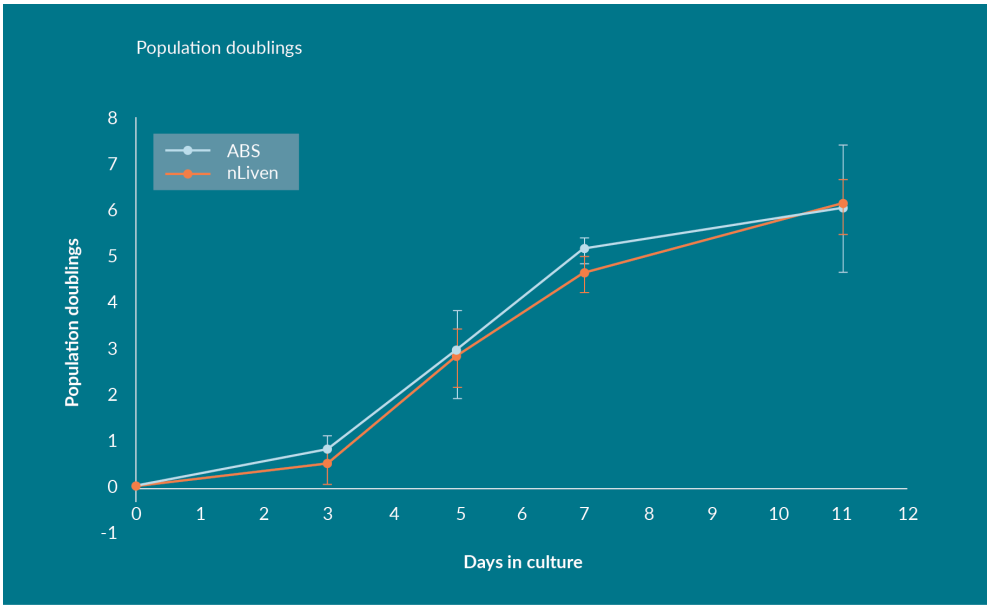
Process consistency is a persistent issue in *ex vivo* manufacture of human primary cells. Especially in autologous therapies, donor-to-donor variability forces a broadening of final product specifications and a looser understanding of critical quality attributes (CQAs). Changing to reagents that reduce variability is a powerful way to improve the understanding of those therapy characteristics. Developers may be able to produce comparable but better characterized therapies since variability was reduced in both studies when the media was supplemented with nLiven PR™ as opposed to human ABS and expansion remained consistent.

Cultures executed at both Sexton and HCATS would typically double between six and seven times during the culture period. Those results show that there was no negative impact on cell expansion by exchanging human ABS for nLiven PR™ and the substitution may result in an increase in total cell yield depending on the homogeneity of the starting cell population.

The percentage of a T-cell population that presents a central memory phenotype is typically negatively correlated to the number of times a population doubled. In two cultures that expanded a similar amount, for instance, the resulting TCM population should also be similar. What was exhibited when culturing with nLiven PR™ in static flasks, however, was a statistically significant increase in this TCM subset compared to media supplemented with ABS (Figure 3; p=0.0006 by T-test). Given similar yields of total cells, the nLiven PR™ conditions are producing a higher number of quality, efficacious TCM cells.

FIGURE 2

Similar average expansion of T cells from three donors (N=3) was observed in the experiments performed at HCATS.



Donor to donor variability decreased by over 50% when the cells were cultured in the presence of nLiven PR™ as opposed to ABS.

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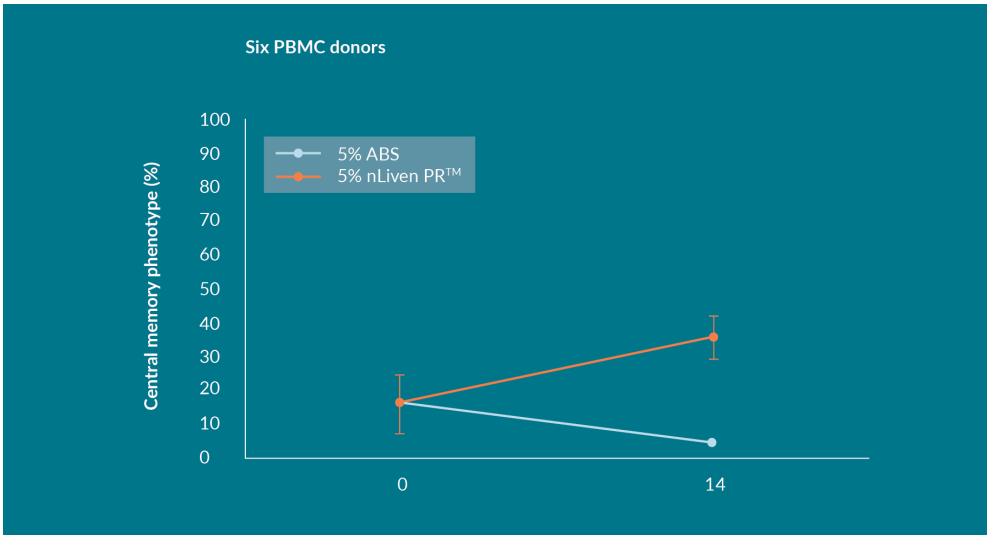
FIGURE 3

Compiled data from 6 independent donor experiments expanded in static flasks showing that nLiven PR™ consistently increases the total central memory T-cell population of PBMCs after 14 days in culture.



The increase in TCM population from using the nLiven PR™ product was statistically significant (p=0.0006 by T-test). The coefficient of variance for the ABS population is 0.25 versus 0.18 for the nLiven population.

This analysis gives credence to the idea that yield should be evaluated as the resultant population of T cells with a favorable TCM phenotype. This quality cell yield could serve as a surrogate for lengthy and expensive *in vivo* potency assays that are out of reach for many groups without a vivarium or for imprecise and target-dependent *ex vivo* potency assays that do not account for the persistence of response. Evaluating culture performance by the final population of TCM may provide a more realistic determination of therapeutic relevance than total T-cell yield.

Regardless of the starting percentage of Central Memory T-Cells by the end of the 14-day culture period approximately 30% of the total population were classified as TCM – this seems to be a consistent and reproducible effect of growing PBMCs in nLiven PR™. Equally, expanding PBMCs in ABS consistently results in a significant reduction of the TCM population.

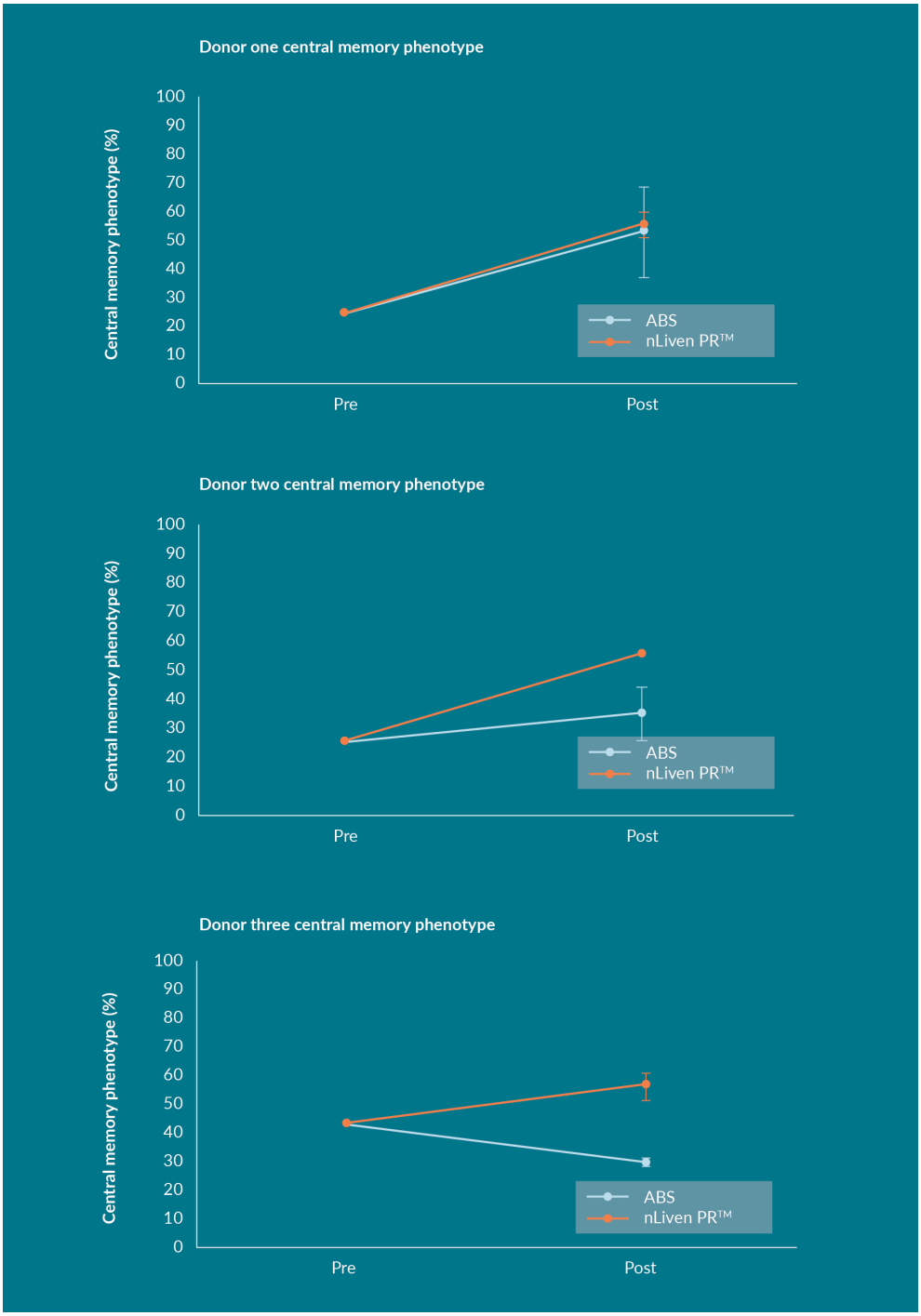
Keeping with the results from Sexton's experiments, the three donors tested by HCATS finished the culture period with about 50% of the T-cell population classified as TCM (Figure 4). This again shows high donor-to-donor consistency with a standard deviation of only 0.2% between donors. Additionally, two of the donors showed statistically significant increases in the TCM population post culture when compared to ABS (Figure 4; donor 2 p=0.02, donor 3 p=0.002 by T-test).

In both Sexton and HCATS studies the T cells cultured with nLiven PR™ saw a net increase in their TCM population after *ex vivo* expansion, which is contrary to the expectation that expanding primary, human T cells drives differentiation toward an effector phenotype. The same result was not duplicated by the cultures supplemented with human ABS. That suggests that the addition of nLiven PR™ selectively promotes the maintenance of a TCM phenotype throughout *ex vivo* cultures.

These independently conducted studies discovered a tendency for the memory phenotypes of the final T-cell population to favor subsets that were correlated with improved therapeutic efficacy and that the resulting expansion of the TCM populations were consistent between donors. In autologous therapies, final product specifications attempt to account for variation in starting material

FIGURE 4

Culturing T cells in stirred tank bioreactors with nLiven PR™ increased the total central memory population in all three donors.



The impact of changing from ABS to nLiven PR™ caused a statistically significant increase in the population for donor 2 (p=0.02) and donor 3 (p=0.002) when analyzed by a student T-test.

collected from individual patients, this factor was mirrored in these studies as Sexton's starting populations were unpurified and HCATS studies utilized $CD3^+$ selected cells. Regardless of starting material the final cell products showed remarkable similarities. Driving down lot-to-lot differences is a major consideration when developing an autologous manufacturing process, and nLiven PR™ is showing promise as a contributing factor to

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improved process robustness. Consistency itself is a valuable trait to develop into the production of an advanced therapy. As a derivative of human platelets, nLiven benefits from a more homogenous combination of biological chemicals than pooled human sera. This difference may be the reason for a more consistent response from primary, human cells.

Typically, the main consideration when evaluating a potential reagent change is the relative yield of viable cells throughout the process, and the results show a comparably high total expansion between the two protein supplements over the evaluated culture periods. However, there is an emerging mindset that focuses on attributes of cell quality in addition to bulk yield. For T cells, memory phenotypes can indicate a therapy's ability to provide a persistent *in vivo* response. The resulting memory phenotypes trend toward the conclusion that TCM phenotypes are promoted in cultures that include nLiven PR™ in the media formulation. Multiple donors from two separate labs had significantly higher TCM populations post expansion. Consistency across a group of donors is another important consideration when investigating if a process impact can be translated into the clinic [7]. When subjected to commercially relevant expansion nLiven PR™ supplemented media resulted in a lower level of variance in total cell yield or central memory phenotypes vs ABS – this indicates more consistent culture conditions that could translate to more robust product manufacturing. The results from the work performed at our labs lead to the conclusion that incorporating nLiven PR™ into T-cell manufacturing processes could lead to a high yield of quality therapeutic cells.

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AUTHORSHIP & CONFLICT OF INTEREST

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