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MANUFACTURING PATHWAY

SPOTLIGHT

EXPERT INSIGHT

Evolving product attributes through the lens of MSC translation

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Mesenchymal stem cell therapeutics have entered the home stretch for market approval, with academic and corporate sponsors reporting mid- to late-phase clinical results which will be highly impactful on commercialization of this product class. How well have translational expectations been borne out? Are there lessons learned from a retrospective perspective that can improve or accelerate successful development? Have baseline product attributes been forecast accurately? This discussion emphasizes the dynamic state of product attributes and integrating bedside-to-bench clinical data, and as well identifies the value of product attributes tied to investment and capital needs to bring these therapies to standard of care.

Submitted for review: Feb 18 2016 ▶ Published: Mar 21 2016

For scientists and engineers involved in translating and commercializing cell therapies, Quality by Design principles are instinctively attractive. By forecasting modality, dose regimen, clinical market and standard of care a profile for successful performance clinically and financially can be a skeleton for decisions to develop. This initial roadmap for creating a target product profile and iterating back to current knowledge creates a technology risk map and defines milestones that de-risk or validate the development approach. These design principles are endorsed by Regulatory agencies, a recent example being the co-published EMA/FDA publication on Quality by Design [1].

At initial planning stages the product profile frequently segregates into attribute streams. Often the first is linked to questions of reproducibility and control for manufacturing scale, cost and consistency. With collective experience in the cell therapy community, solutions to prior hurdles have become templates, and a number of unit operations have been reduced to plug and play solutions for more common path cell and gene therapies. This is outlined in a position paper published by a consortia of investigators collaborating through the International Society for Cell Therapy [2].

A second crucial block of attributes are those linked to biology and potency, where both *ex vivo* and *in vivo* testing should confirm basic confidence in mode of action (MOA). Limited high sensitivity tools exist for fate mapping and

persistence of donor populations in the pre-clinical setting and this has hindered dose planning for clinical entry. Development of product attributes associated with potency has been well described in a recent review [3].

A third pillar of attributes contains those linked to biodistribution and surrogate assays to detect *in vivo* potency. These latter attributes are often initially understudied for lack of technology or until clinical data and exploratory datasets provide a feedback loop.

Decisions for a therapeutic to enter the development pipeline are based on risk assessment for being able to successfully mature initial baseline technology to meet target attribute thresholds. This is the case for technology emerging from the academic community into a commercial start-up, or for progression of programs within a large pharma or healthcare environment.

WHAT IS THE LEGACY FROM HEMATOPOIETIC THERAPIES ON MSC DEVELOPMENT APPROACHES?

The practice of cell therapeutics was launched from transfusion and transplant medicine, with our current principles appropriately and strongly drawn from the hematology community. Over the last 15 to 20 years significant clinical experience has been gained from translation of adherent bone marrow-derived cells, multipotent mesenchymal stromal cells (MSCs), representing a new cell therapeutic model for *ex vivo* expansion of cells with allogeneic potential and commercially modeled as a biologic.

The early clinical practice for MSC expansion utilized common tools such as those practiced in the hospital stem cell laboratories, and therefore shared a common practice for standards involving ancillary materials, facility management and culture hardware. Many of the product attributes required to meet pass/fail criteria for blood cell processing (e.g., viability >70%) were directly captured and implemented. This was largely highly beneficial for the industry.

Similarly the paradigm to associate biological potency with phenotype was drawn from the hematopoietic community, where use of surrogate hematopoietic stem cell (HSC) phenotypic markers (e.g., $CD34^+$; $CD133^+$) correlated with transplant outcome. Anticipating a similar logic within the MSC community an approach was taken to adopt a phenotypic potency assessment but combined with *in vitro* lineage differentiation assays as a surrogate for lack of clinical experience [4].

Retrospectively this phenotypic characterization carried complications along several dimensions, the first being a consequence of *ex vivo* expansion and variability of MSC phenotypic markers. It has been clearly demonstrated that the isolated progenitor for bone marrow MSC undergoes significant phenotypic shift from isolation and exposure to tissue culture substrate and media [5]. This introduces the complexity of associating *ex vivo*-derived phenotypic markers with potency.

This is further complicated by the absence of engraftment or serial transplant models for *ex vivo*- expanded MSC. Unlike the hematopoietic space where retention

DOI: 10.18609/cgti.2016.010

of stem or progenitor cell potency can be demonstrated biologically, MSCs have frequently not shown retention in tissue biopsy and when assayed have not been re-isolated from bone marrow aspirates of transplanted patients [6,7]. As a consequence, there has been limited association with a phenotypic product attribute linked to biological potency. Instead, phenotypic variability has been an attribute seized on for intellectual property or identity and not functionality. It is not surprising that in efforts to optimize MSC production using growth factors, gas conditions, adherence substrata, bioreactors, and restricted and variable serum lots has resulted in myriad overlapping phenotypic distributions. These distributions create the product attributes equivalent to a financial "valley of death" – an environment where product attributes no longer relate to the *in vivo* progenitor or counterpart, and the product hasn't advanced in clinical development far enough to create a patient assay feedback loop to correlate a phenotype to a response. Yet this is the stage at which critical manufacturing decisions are routinely being made and would strongly benefit from a rapid in-process phenotypic confirmation of potency. This reinforces that the key application of MSC phenotypic product attributes is for manufacturing consistency and detecting the absence of unwanted cell residuals, rather than a utility for potency.

NEW PARADIGMS FOR POTENCY ATTRIBUTES?

Initial definition standards for MSC included *in vitro* mesenchymal lineage differentiation assays as a second component in addition to phenotype [4]. At the time these biological assays reflected the primary ongoing translational activity, notably in bone marrow transplant and orthopedics, and as well the anticipation that these *ex vivo* populations would engraft and contribute to new tissue formation.

Over time it has become clear that the primary therapeutic modality for infused MSC is to elaborate trophic factors which modulate inflammation/ischemia and immunity as well as stimulating angiogenesis and tissue repair [8]. As a consequence MSCs have been used in a diverse range of clinical conditions where the biological potency is unlinked to mesenchymal cell fate [9], and therefore those initial lineage differentiation attributes are not appropriate.

The guidance produced from the FDA regarding potency assay development advises on testing a matrix of biological assays [10], which may be very relevant advice for a platform product like MSC. It would certainly be logical to qualify an MSCs master cell bank for multiple indications and optionality. There is also the implicit benefit that capturing a larger range of pathway attributes can identify failure nodes and valuable dependencies for in-process improvements.

TAKING A PATHWAY APPROACH TO IMMUNOMODULATION

An important consideration and in fact a significant bottleneck in MSC potency assay studies is the lack of validatable assay formats for cell-based assays. In order to develop a qualified assay to meet

manufacturing and lot release criteria careful attention must be made to assay components, format and gold standards. This includes adopting established core assay formats such as ELISA or flow cytometry and avoiding variable test materials such as primary cell materials.

As an example, perhaps the most employed MSC potency assay is a derivative of a mixed lymphocyte reaction which involves stimulating T cells to proliferate and then measuring inhibition. Yet each time the assay is run a different test material, donor T cells, are collected and tested. This assay is considered highly reliable as a qualitative assay for immunomodulation but highly variable and prohibitive to implement towards commercialization.

At the 2015 ISCT Annual Meeting in Las Vegas, USA, a productive workshop was held by the ISCT MSC committee. The topic of the workshop and the resulting position paper was quite creative in forecasting solutions around current assay technology roadblocks [11].

The principle for the approach was to step up a level and interrogate the pathways through which MSCs are known to immunomodulate an activated T cell response, for example through prostaglandin synthesis, tryptophan metabolism, iNOS regulation. These pathways have been shown to be induced when MSCs are exposed to an inflammatory environment or primed, and therefore can be detected in MSCs following IFN-γ stimulation. The recommendation from the ISCT MSC committee is to turn to a standard immunodulatory assay which confirms that an MSC population upon IFN-γ stimulation will activate these pathways to a statistically prescribed pass/fail threshold.

This is a useful and forward looking solution to a series of complex cell-based assays. There is an interesting implication to this approach which should be therapeutic to the MSC community itself, which is the re-casting of MSC potency into a few broad "genus" categories based on biological and not phenotypic properties and avoiding the artificial categorization using individual "species" markers.

Organizing MSCs according to broad functional categories has the consequence of classing most products into a common bin, with the commercial implication that this is a product class of generics whose distinction to date has been largely driven by artificial single gene phenotypic identities.

One can envision a downstream effect as a consequence, in order to gain clinical label distinction or intellectual property positioning for investment. This will drive innovation towards production methods sustaining new pathway responses, or towards gene therapy or epigenetic regulation to create new product attributes.

HOW DOES BIG DATA HELP US?

In the above example an advocacy case can be made for moving to top level pathway response as a means of assessing potency potential. An extension of this argument would lobby for the collection of large datasets of descriptive 'omics biology for an MSC culture platform. This would then define the potential and classification of the product, and limit the value for interrogation of a few phenotypic or pathway markers in terms of

DOI: 10.18609/cgti.2016.010

potency. However, regulatory agencies do not find large datasets useful in the absence of tight correlation to function and a reductionist path to correlate attributes with function.

To date large transcriptional profiling exercises have proven of interest in dissecting differences between MSC and MSC-like cells derived using alternate culture conditions but have been less useful in building a fingerprinting screen for identity or function [12,13]. This is in part due to the analysis approach being primarily statistical, wherein principle component analysis identifies a set of genes whose quantitative difference between test samples is highest and therefore the basis for distinction. The biology of members of this gene block is not inherently linked, and hence such a fingerprint doesn't necessarily call out the core biology desired in tracking change.

New technologies in epigenetics and RNA metabolism bring alternative approaches. microRNAs (miRNA) in particular pose an interesting solution to pathway analysis but formatted for insightful profiling on validated assay platforms. Crabbe *et al* report on an integrated bioinformatics approach to measure miRNA associated with identity and function profiling of MSCs and multipotent adult progenitor cells (MAPC) [14]. Because each miRNA is known to regulate and influence multiple mRNAs in a pathway context, miRNA expression can be a master regulatory snapshot of cell functionality. This case illustrates the value of integrating composite epigenetic measurements in building a biologically justified attribute panel. For example, analysis of gene methylation events suggested differential splicing or RNA processing at a locus that could also be interrogated for miRNA correlations or changes in transcriptional abundance.

FROM BEDSIDE TO BENCH

Early clinical studies address safety and tolerability and provide a first snapshot for donor product distribution and persistence. These studies also provide a first measurement for cell persistence and durability of response. Cell persistence is an important component of a Target Product Profile (TPP), given the implications for dose or repeat dosing. However this is often only studied retrospectively when clinical data allows for stratification of donor product and associated properties.

The translational practice of MSCs ranges from autologous to allogeneic donors, and from single to multiple donors used in allogeneic clinical studies. Studies to determine product attributes linked to clinical development and donor variability have not been informative; however correlation has been made to early passage MSCs correlating with complete clinical response where late passage MSCs do not [15]. This may be an example of a potency attribute linked to tissue biodistribution or migration to which the MSC community has been blind due to in part to a lack of surrogate assays for *in vivo* pharmacokinetic properties as well as the absence of clinical experience and limited cycles of bedside to bench iteration.

PRODUCT ATTRIBUTES & COMMERCIALIZATON

These past 15 years in the MSC community have largely been about

belief; convincing ourselves that we can harness the biology we witness in the laboratory into a new medicine; and convincing the capital community that this merits investment. For better or worse, the patients need no convincing as evidenced by the huge global demand for treatment. As a consequence the emphasis on MSC product attributes has been around mechanism of action and building correlation between clinical response and properties we can measure *in vitro*.

In the last 10 years significant emphasis has been placed on scalable manufacturing, and attributes have evolved to encompass extended population doublings and production campaign targets are now implicit. And over the past 5 years clinical results are becoming public, thus initiating an informative feedback loop on our understanding of MSC clinical modalities. These composite MSC clinical results are showing substance and should concrete a foundation for this industry segment.

As previously stated, early development decisions are based on a risk assessment for meeting a TPP, figuratively linking baseline to the end of the rainbow. In retrospect, from the MSC community experience it seems evident that another attribute stream could have been applied as outlined in **Box 1**.

Translating MSC therapies has moved to the stage of mid-phase clinical data coming forward with criticality for bedside-to-bench validation of attributes forecasting clinical response. This platform of living medicines has tremendous potential for patients and by continuing to refine our development vision we can be confident of their progression to standard of care. As the MSC community adapted and learned from the hematology/oncology community, there is now an opportunity to refine how we define and utilize MSC product attributes as a new paradigm for the next generation of cell therapies growing in the regenerative medicine space.

BOX 1

1. Validation of business model and the class of investors in therapeutic space
   - defining an attribute for early financial discussions that places the product in an optimal product class
   - identifying a technology attribute which is seen as high value or represents an exit point for the investor class
2. Regulatory path feedback showing product feasibility
   - defining regulatory feedback which de-risks the development path
   - exploring regulatory feedback in geographies supporting accelerated approval
3. Accessibility to non-dilutional capital by grants and development agencies
   - create product attributes meeting public sector development support targets for valorization
   - utilize platform product attributes to target orphan indications or advocacy group supported development funds
4. Timing of high value experimental inflexion points in platform/product
   - determine technology attributes of a product platform which can demonstrate dimensionality or high value concepts and use for financing events independent of longer term clinical development advances

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The author has previously been employed by Osiris Therapeutics Inc and Athersys Inc, therapeutic sponsors in the adherent adult stem cell space. The author holds stock options and patents assigned to these entities. No writing assistance was utilized in the production of this manuscript.

DOI: 10.18609/cgti.2016.010

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