International community consensus standard for reporting derivation of human embryonic stem cell lines

**Emma L. Stephenson^{1,2},
Peter R. Braude^{2,3} &
Chris Mason^{1†}**

[†]Author for correspondence

¹Advanced Center for
Biochemical Engineering,
University College London,
London, UK

Tel.: +1 207 679 0140

Fax: +1 207 209 0703

E-mail: chris.mason@ucl.ac.uk

²Department of Women's
Health and Division of
Reproduction and
Endocrinology, King's College
London, London, UK

³Assisted Conception Unit,
Guy's and St Thomas'
Foundation Trust, London,
UK

**Karen Rowland,
Graphics Editor**

Future Medicine,
Unitec House, 2 Albert Place,
Finchley Central, London,
N3 1QB, UK

Tel.: +44 208 349 2033

Fax: +44 208 343 2313

E-mail: k.rowland@futuremedicine.com

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The development and implementation of new methods in human embryonic stem cell (hESC) research is fraught with difficulties, not least of which is the highly variable reporting of the number and quality of embryos used to derive hESC lines. Without a clear minimum information convention among the derivation teams, understanding and comparing derivation methods and their potential impact on the resulting stem cell line will continue to be extremely difficult. In order to address this issue, we consulted international teams regarding the implementation of a minimum information convention for derivation with the aim of universal use, data collection and central analysis, followed by a multi-author publication. The responses demonstrated overwhelming support for such a system; over 90% of the respondents agreed that a universal standard for reporting the derivation of hESC lines was essential as part of the international effort to advance the field efficiently, and over 87% plan to use this standard and share collected data in Spring 2008 for central analysis and public dissemination. A number of future steps are planned in order to ensure that this standard evolves with the field and remains relevant and up-to-date. Our aim is to incorporate these data within existing international initiatives aimed at improving derivation standards. This article is an open-access publication in order to make the convention freely available to the international community and encourage universal participation.

In November 2006, we published a proposal for a universal minimum information (MI) convention for the reporting on the derivation of human embryonic stem cell (hESC) lines [1]. The requirement for standards in derivation and stem cell science originates in part because the field is new and therefore needs structure. Also, it involves expertise from diverse scientific backgrounds, from embryology to regenerative medicine. Standards function as agreements between people in order to enhance information exchange and mutual understanding [2]. The challenge faced with embryology and stem cell derivation is the attempt to overlay discretely defined standards on a continuous spectrum of developmental variance. However, the use of MI datasets in other biological fields has shown that standards can greatly help avoid misunderstandings and duplication of work and aid clear, precise communication [3,4]. Furthermore, the adoption of standards enables researchers to reproduce each other's results, to collaborate more productively and to increase efficiency [2]. Indeed such is the growing recognition of the need for standards, that the introduction of new article categories in journals are based on the opinion that standardization of experimental methods is a prerequisite for high-level science [5]. This drive for standardization can be seen clearly

in many areas of stem cell and regenerative medicine. Loring and Rao recently proposed that a set of shared standards should be available in order to characterize hESCs unambiguously in multiple laboratories [6], and the conclusion of the UK Department of Trade and Industry (DTI) workshop entitled *Standardization to support the Regenerative Medicine Industry* was that '...the industry requires a higher level of clarification with respect to legislative and standardization issues for cell-based therapies' [101].

In order to start off this process in hESC derivation, we conducted a thorough survey of the majority of the international derivation groups and requested their opinions and comments on our proposed MI convention [1]. Using the analysis of the data obtained from their responses, we now describe a community consensus on the minimum data currently required to create a meaningful standard for reporting the derivation of hESC lines.

Methods

Data collection process

There are a number of universally agreed key steps that are required to initiate, develop and maintain a biological standard [7–9]. These steps are laid out in **Figure 1** and all have been addressed in the consensus process.

Figure 1. Formation of the standard: universally agreed key steps.

Step 1: identify the scope of the information that needs to be captured in the standard

In the original manuscript ‘Proposal for a universal minimum information convention for the reporting on the derivation of human embryonic stem cell lines’ published in the November 2006 edition of *Regenerative Medicine* [1], we reviewed extensively the existing relevant derivation literature and drew the conclusion that there was no universally agreed method for reporting the quality and type of human embryo used as starting material for hESC line derivation. Whilst many groups had published their hESC derivation data and protocols, few had stated in their manuscripts the complete essential underlying metadata with respect to their source material to enable reliable comparisons to be made. However, it is acknowledged that this lack of an accepted and established reporting system results in several hurdles for

stem cell researchers. For example, it is almost impossible to ascertain from the literature a true efficiency for deriving a hESC line.

With the help of a small number of leading hESC derivation and standards experts, a draft standard was produced that included a MI convention dataset together with a detailed grading system for blastocysts used in hESC line derivation.

Step 2: develop a simple and easy-to-read framework for the information that is needed to be included in order to develop a MI checklist or specification

The draft proposal was written as a manuscript that included a table that outlined the MI data deemed by the authors, together with guidance from a small group of international experts, as either ‘essential’ or ‘desirable’ in publications that report the use of human embryos for stem cell research. An embryo grading system (Figure 2) was presented in a series of color drawings with legends. A number of photographic examples of the various grades of embryo were also collected together and labeled appropriately to facilitate comparison with microscope images (Figure 3).

Step 3: make the draft MI specification freely available

The draft proposal was published as the lead editorial in the Medline-listed journal *Regenerative Medicine* in November 2006 [1]. The Editorial Director kindly provided the draft proposal on the journal’s website [102] as a free-of-charge open-access download in portable document format (.pdf). A substantial number of full-color paper copies were provided that were posted personally and delivered or distributed at a number of national/international conferences, including in Europe, Asia, Australia and North America.

Step 4: develop a consensus view of the MI that needs to be reported

The editorial contained several requests for feedback including the following in a large text box: ‘Interested parties are encouraged to forward their views and comments as soon as possible in order that a consensus document can be published in the not too distant future’. However, because of the essential requirement to achieve a high-level consensus final document, the full-color copies of the publication were sent to 40 renowned international hESC derivation or

stem cell culture groups. Included was an explanatory letter plus a two-page questionnaire consisting of multiple-choice questions regarding the data, and also fields for free-text comments with the request to complete the questionnaire and return by fax. In order to maximize the final response rate, the questionnaire was both emailed and followed up by personal contact. All comments and suggestions have been discussed and incorporated where appropriate. All the contributors are gratefully acknowledged at the end of the paper.

"The UK Stem Cell Bank supports the stem cell community through provision of well characterized and ethically sourced human stem cell lines and promoting the dissemination of best practice in the technical issues relating to the use of the cells. It is important therefore that we adopt and promote consensus on key issues of quality for stem cell lines. Adoption of the authors' proposal, on the establishment of criteria for the evaluation of embryo quality, by the stem cell research community in general would provide the kind of consensus that the UK Stem Cell Bank would accommodate and promote in the evaluation of cell lines in its collection."

– Glyn Stacey, Director, UK Stem Cell Bank.

Step 5: circulate widely the agreed protocol in order that the metadata can be correctly incorporated into reports, publications, grant applications and the general dissemination of data

This process begins with this publication. Once again, the Editorial Director has kindly agreed to provide free open-access downloads. This publication will also be distributed widely to hESC derivation laboratories together with details of how to access the data collection form, which will be available as a download from both the Future Medicine (http://www.futuremedicine.com/doi/suppl/10.2217/17460751.2.4.349/suppl_file/I_CSS.pdf) and UK Stem Cell Bank (www.ukstemcellbank.org.uk) [103] website. However, it must be noted that the form is only a suggested tool to aid collection of all the necessary information; it is not an essential prerequisite of the standard. In addition to stem cell scientists, the paper will be circulated to stem cell banks, standards agencies (including the British Standards Institute), networks including the International Society for Stem Cell Research who have a specific interest in developing standards, and regulatory authorities including the UK Human Fertilization and Embryology Authority (HFEA). A number of groups have already adopted this draft standard for the reporting of all their hESC derivation.

Step 6: standards are an ongoing process, therefore care must be taken to ensure that they are developed as the field progresses and new technologies evolve

This final step is of paramount importance in order to ensure that the standard does not quickly become out-of-date and obsolete. Therefore, we plan to:

- Provide advice to others to help facilitate the universal adoption of the standard;
- Use the standard as a means of gathering derivation data from the community and feedback regarding the included categories;
- Approach the community again in Autumn 2008 to obtain a new consensus based on their experience with the current standard and in the light of scientific advances in this rapidly advancing field.

Data analysis

The multiple-choice section of the questionnaire requested that the respondents judge the datasets in the proposal and indicate whether they considered such information 'essential', 'desirable' or 'not required' in publications and data collection regarding hESC derivation. The initial analysis was a simple vote count of these responses and a percentage calculation to determine the consensus view of each dataset. Following this, in collaboration with experienced statisticians, a linear weighting system for the data was devised and piloted by a small subset of the sample group. The response 'essential' was weighted as +3, 'desirable' as +1 and 'not required' as -1. Each response was tallied and summed appropriately. This weighting system allowed the categories to be ranked with respect to the spread of responses. In addition the respondents were asked to answer specific questions confirming the use of the convention and the provision of data. The responses were counted and presented in percentage form.

Results

From the 40 groups targeted with this survey, plus a small number of voluntary contributions from interested parties, we received 31 responses. The results from the simple vote count and percentage calculation are shown in Table 1. The vast majority (17 out of 18) of the datasets considered in the survey were categorized as 'essential' for collection by the participants. The remaining dataset (1 out of 18) was judged as 'desirable'. Overall, there were very few

Figure 3 (above). Photographic examples of inner cell mass (ICM) grades A–E.

Photographic examples of the ICM appearance represented in diagrammatic form in Figure 2. These pictures demonstrate ICM grade A–E only, and do not consider expansion or trophectoderm appearance. It is important to scan through every plane of the blastocyst to confer an accurate grade. ICM indicated by arrow.

Figure 2 (left). Classification system for human embryonic stem cell (hESC) derivation.

Diagrammatic representation of the grading system for blastocysts used in hESC derivation. Column 1 shows expansion status only; the ICM and trophectoderm are standard to highlight the variable. Similarly, Column 2 shows ICM appearance only, and Column 3 trophectoderm morphology only.

Table 1. Outcome of vote count.			
Data set to be reported for each derivation project	Essential data (%)	Desirable data (%)	Not required (%)
Total number of embryos transferred to the research project	70	13	17
Stage of embryos used	77	23	0
Number of transferred embryos developing to blastocyst	65	29	6
Number of blastocysts suitable for use	74	19	7
Day of development of blastocysts used	77	23	0
Grade of all blastocysts used	68	26	6
Number of ICMs obtained	77	16	7
Method used to isolate ICMs	90	10	0
Number of ICMs that attach to feeder layer/ECM	60	30	10
Number of days until appearance of stem cell-like outgrowth	35	55	10
Number of stem cell-like colonies	47	40	13
Number of hESC lines established	97	3	0
Known genetic disorder (if PGD)	81	19	0
Which proprietary IVF media used	61	39	0
Gas phase employed	68	26	6
Source of embryos if fresh	68	32	0
Hatched naturally or ZP removed	55	35	10
Sex	55	42	3

ECM: Extracellular matrix; hESC: Human embryonic stem cell; ICM: Inner cell mass; IVF: In vitro fertilization; PGD: Pre-implantation genetic diagnosis; ZP: Zona pellucida.

votes for the ‘not required’ choice, and indeed, none of the datasets fell into this category in the consensus results.

After applying the weighting system to the responses the datasets were ranked accordingly. The outcome is shown in **Figure 4**.

Replication of the specific questions asked on the feedback form and the percentage of affirmative replies is shown in **Figure 5**.

Discussion

Data analysis

The outcome of the survey revealed the community opinion that all the datasets suggested for collection are of great interest. None of the sets were rejected, indeed 17 out of 18 were considered essential for inclusion when reporting derivation. The rank order indicates those categories considered to perhaps have the greatest effect on outcome of derivation attempts, hence essential for collection and analysis. The top ten ranked variables include method used to isolate ICMs, stage of embryos used, proprietary *in vitro* fertilization (IVF) media used and grade of all blastocysts used. Several further datasets were suggested

in the comments that are discussed in detail below. In these early days of derivation attempts, it is prudent to collect all the data available at each stage of the derivation process as the inter-relationships between the culture variables and the effect on the successful outcome is not yet fully understood.

The excellent response rate to the survey (31 out of 40) and the content strongly indicates that the majority of the derivation community consider the need for standardization a pressing issue. Indeed, over 90% of the respondents agreed that a universal standard for reporting the derivation of hESC lines is essential as part of the international effort to advance the field as efficiently as possible. In order to obtain any significant data regarding the parameters involved in derivation, results must be shared, pooled and centrally analyzed, since the number of lines derived in each individual laboratory is insufficient to draw meaningful conclusions. With over 87% of the surveyed groups agreeing to use this standard and over 85% of those to share collected data in approximately 9 months’ time, we are confident that we shall soon see the benefits of collecting such standardized data.

Figure 4. Rank order of dataset following weighting.

Specific comments on the dataset

Total number of embryos transferred to the research project

The request to report the number of embryos reaching each stage of the derivation procedure was to facilitate better comparisons between protocols and to permit a more accurate calculation of the efficiency of derivation. While the majority (70%) of respondents judged this data to be essential, even mandatory, prudent concerns were expressed. The most common observation was that the exact point at which teams define the transfer of embryos to a research project is

inconsistent between groups and often unclear in publications. For some, it is the total number allocated to research, disregarding whether or not they were used (intention to derive) and, for others, only those embryos deemed suitable for use at the required developmental stage. Similarly, the point was made that when cryopreserved embryos are used, not all of them survive the thaw step; allocation to research prior to thawing would include nonviable as well as viable embryos. Clearly, both these variations will have significant implications when different groups report their efficiency of derivation.

Figure 5. Standard consensus.

Some said they would find it difficult to specify when an attempted procedure was sufficiently different to justify reporting as a separate project. Others were concerned that any new procedure would have an inherent learning curve, with the potential for a period of lower efficiency that is not due to the technique itself. All of these issues are valid and reflect the value of the survey process. We feel confident however, that the collection of all the data categories suggested here will enable groups to calculate efficiency using any denominator that they choose as an assessment of their work, as long as they are precise. The number of fresh embryos donated to research should encompass all those received from a patient with informed consent that are not transferred in the fresh cycle nor cryopreserved for their later use. For supernumerary cryopreserved embryos donated for research use, the number recorded should be the total before thaw. Subsequent data that record the number of embryos developing to blastocyst and the number of blastocysts suitable for use in derivation will then allow calculation of developmental efficiency as well as outcome, with respect either to total number of embryos donated or to total number used. Reporting all of this information will also enable monitoring of trainee improvement, ensure continued proficiency of experienced staff and act as an early warning system if external influences are having a detrimental effect on derivation procedures.

Stage of embryos used

Being able to define the stage of pre-implantation development from which it is most likely that a line will be established is important information and was judged to be in the 'essential' category in the consensus. There have been reports of successful derivation from morulae [10] through to day-8 blastocysts [11] and, more recently, from single blastomeres at the cleavage stage [12], but currently the success and efficiency from each stage has not been established. Central analysis of a large collected dataset including stage, quality and outcome will enable researchers to begin to make these distinctions.

Number of embryos developing to blastocyst

Recording the percentage of embryos allocated to research that develop to the blastocyst stage enables feedback to IVF clinics on their culture systems, especially if they differ between culture for therapy and research. It was commented that this rate will vary greatly depending on the source IVF lab. Indeed for any stem cell derivation group, the backing of a committed and successful IVF center is key to obtaining good-quality, progressive embryos for research. This information is valuable when making global estimates of the number of embryos that might be needed to generate lines.

Number of blastocysts suitable for use

When analyzing the derivation literature for the initial proposal, it became evident that some groups select blastocysts for derivation attempts

based on their own individual criteria. Multiple respondents commented that if blastocysts are defined as ‘unsuitable’, the reasons for excluding them must be given. Indeed several groups commented that they would not reject a blastocyst of any quality in these initial stages of basic research and development. Certainly in our own derivation attempts, all embryos that develop to the blastocyst stage are used; we simply tailor the derivation method attempted depending on the morphology. Acknowledging the fact that this assessment of whether or not to use an embryo may be subjective, perhaps the adoption of common suitability criteria for different methods of derivation that depend on the morphology and development of the embryo might be useful, but are beyond the scope of this MI convention.

Day of development of blastocysts used

Information regarding the day of pre-implantation development that blastocysts are used and the resulting outcome will not only reflect on the culture systems employed, but will also inform whether there is a ‘window of permissiveness’, as is the case in derivation from murine blastocysts; that is, whether there are specific days of pre-implantation development from which the derivation of hESCs is more likely, and that attempts outside this window are less likely to be successful.

Grade of all blastocysts used

It became clear when researching the literature that very rarely did teams report the grade of blastocysts used in their derivation attempts. Even when reported, the grading systems used were not uniform. Hence although tentative comparison of results might be possible by studying the particular systems used, it would be more accurate, consistent and reliable to implement one easily available system in order to establish the range of blastocyst quality that gives optimum derivation efficiency. This will act as a guide for research groups, allowing them to monitor progress or changes in protocol against a known expected outcome. The grading system proposed, and reproduced here from the original document as **figure 2**, has been based on advocated criteria after many years of clinical experience, but tailored specifically to hESC derivation. In the future, the IVF field might move away from simple observation and grade allocation, to a system that uses automated and case-based reasoning. Jurisica and colleagues recently reported the application of

automated image analysis to evaluate morphology and developmental features of oocytes and embryos in IVF [14]. Although humans can analyze images more flexibly, computer vision techniques make the process more objective and precise, which when combined with case-based reasoning, allows feature extraction, indexing and analysis. As with standards enabling meaningful data analysis, such a database of analyzed embryo images will enable more accurate correlation of specific morphologic features with outcome, both clinically and in research. No two hESC lines are identical, and knowing the quality of the starting material may help elucidate the underlying differences.

Method used to isolate inner cell masses

Successful derivation of lines has been obtained following inner cell mass (ICM) isolation using a number of methods: immunosurgery, mechanical disaggregation, laser isolation and even plating of the whole blastocyst followed by mechanical separation of hES-like cells. Currently, it is not known which of these methods is most efficient or whether the protocol should be dictated by the morphology of the blastocyst. By reporting both the grade of blastocyst used and the method for derivation, these relationships can begin to be unpicked. Reporting whether or not ICMs are isolated is also important to some regulatory agencies. In the UK, the HFEA ruling that embryos can only be kept in culture for 14 days, or until the appearance of the primitive streak (whichever is sooner) [13], begs the question as to whether whole embryos once plated and grown beyond this time would fall foul of the ruling despite the cyto-architecture and 3D structure of the embryo being irreversibly lost.

Number of ICMs that attach to feeder layer/extracellular matrix

Not only will this provide information about ICM quality, culture systems and technical proficiency, but recording the day and proportion of the ICMs that attach may also provide some data on the role of anchorage in success of derivation. A slight modification to the wording is necessary however. In the original draft, the dataset was ‘number of ICMs obtained’ followed by ‘number of ICMs that attach to feeder layer/extracellular matrix (ECM)’. However, some groups may isolate ICMs but not use them for derivation; for example, ICMs may be used for gene analysis.

Therefore, in light of the collected data and comments, the former category has been changed to ‘number of ICMs placed on feeder layer/ECM’.

Number of days until appearance of stem cell-like outgrowth

Although it is not always easy to observe definitively the first appearance of stem cell-like cells in culture, the variability in the number of days before this feature is noticed makes this category intriguing. Although the observation may be too arbitrary ever to draw firm conclusions regarding the relationship with embryo quality and outcome, the majority of responses (55%) rated it as desirable, indicating that this is an area of interest for researchers.

Number of stem cell-like colonies

Occasionally, stem cell-like cells are propagated in culture, perhaps reaching very early passage, but do not become established. This could be due to degradation in culture, for instance, as a result of replication arrest or contamination with microorganisms. The intention here is to record those outgrowths that at first appear to have potential. It should be stressed that it is important to report these putative colonies, as they add to the valuable information to be extracted from embryo number and quality data, as well as comparisons between protocols and assessing the impact of external influences, such as laboratory conditions.

Number of hESC lines established

The exact point at which a hESC line should be deemed established is arguable. This area was discussed in the original publication [1]. Not surprisingly, this category received almost universal comment. Although some reports use a defined passage number, most would consider a line established only when it has been characterized, successfully cryopreserved/thawed, retained stable characteristics after extended culture and ultimately banked. Although beyond the scope of this convention, the criteria to be used to define a line is an issue of paramount importance and is being addressed by the International Stem Cell Initiative (ISCI), and the field awaits their recommendation. Until clear and uniform standards have been established for what constitutes a hESC line, it is suggested that each group defines the criteria they use for each report of an ‘established’ line.

Gas phase employed

The vast majority of centers culture human embryos and hESCs in 5% CO₂ in air (21% O₂). As the assisted reproductive technology (ART) field moves towards accepting the potentially detrimental effects of free radicals on embryos and implements culture at more physiological oxygen concentrations (1–5%), it will become essential to record this variation in hESC derivation attempts and outcome. This should be reported for both embryo culture and stem cell culture if it differs. Along with gas phase and proprietary IVF media used, it is acknowledged by the consensus responses that other culture conditions and variables are certainly worth noting, such as whether the embryos are cultured in groups or individually prior to being assigned to research, whether derivation attempts are in microdrops or well plates (and their volume), or in 2D or 3D culture. While appreciating fully that the feeder layer/ECM and media used will have a significant influence on the outcome of derivation attempts, these details are beyond the scope of this particular MI convention. Our aim has been to focus effort on the embryological culture and manipulation that we hope will complement the admirable work of researchers concentrating on feeder/ECM and media comparisons in culture [15–18].

Hatched naturally or zona pellucida removed

The hatching status of blastocysts on each day of growth gives an indication of blastocyst quality and developmental capacity. Although hatching status is inherent in the proposed grading system, it has been rightly suggested that the mode of zona pellucida (ZP) removal could affect outcome. Various methods of enzymatic or chemical treatments have been reported to affect the surface properties of the plasma membrane and the microvilli [19] therefore this heading has been modified to read ‘hatched naturally or method of ZP removal’. It will also indicate if the zona has been breached previously (e.g., for pre-implantation genetic diagnosis biopsy).

Karyotype

Arising repeatedly was the comment that the reporting of a detailed karyotype (including ploidy status) and the passage number at which the analysis was performed should be included, not just the sex of the line. Determining the cytogenetic and epigenetic status of hESC lines

should be a standard component of the repertoire of assays performed to fully characterize a new line. Given the high occurrence of aneuploidies observed in human embryos [20], it seems surprising that upon initial characterization, the vast majority of hESC lines are genetically normal. However in 2004, Draper and co-workers reported alarming data to suggest that lines should be regularly karyotyped while in culture, as they observed karyotypic changes involving the recurrent gain of chromosomes 17q in three independent hESC lines on five independent occasions, and the gain of chromosome 12 was seen occasionally [21]. Questions remain however, particularly regarding the type of analysis to be performed; whether standard G-banding is sufficient, or whether comparative genome hybridization (CGH) is more appropriate as chromosome banding cannot detect microdeletions, amplifications, point mutations nor minor rearrangements that may also affect growth, survival or differentiation. How often such analysis should be performed is another area for debate, as is an agreement on what proportion of transformed cells in the sample constitutes an abnormal result. In light of such comments, we have modified our dataset from the simple reporting of the sex of the line to a detailed karyotype. It is recommended that given the current lack of a standard protocol, each group defines the method used and at which passage the analysis is performed.

In addition to the comments directed specifically at the data categories there were numerous valid, insightful remarks about other datasets that could be added, or general suggestions to improve the reporting of derivation. Several of the respondents suggested that photographs with scale bars would be useful. Photographs taken of the embryos on consecutive days of development, of ICMs after immunosurgery and of hESC colony outgrowth could be very helpful in analyzing and comparing the results of derivation attempts from various laboratories since opinions on morphology can be subjective. It is also important to record and report the dates of data collection to monitor development and progress of techniques and ensure complete yet nonoverlapping data. As techniques develop, success will increase and this could distort the figures. Knowing whether the operator had previous experience with embryology or derivation would be similarly valuable. In addition, information regarding

the quality status of the laboratories will be important for future therapeutic applications, that is, whether the laboratory has achieved good manufacturing practice (GMP)/good laboratory practice (GLP) accreditation, or if the culture of embryos and stem cells is under general or particular (e.g., EU Tissue and Cells Directive) IVF standards. In the same vein, virology screening results of donors will need to be a consideration as the field moves toward clinical application of these cells.

Being able to obtain further information from the clinical IVF end of the process is a privilege that those derivation teams working closely with the IVF center should take advantage of. Recording the etiology for the patient's cycle, that is, whether the cycle is due to male or female infertility factor, whether the treatment was IVF or intracytoplasmic sperm injection, and whether transfer of embryos in the same cycle resulted in a successful biochemical/clinical pregnancy and/or live birth, would all be interesting data. Similarly, knowing the ethnicity of the patients may reflect the inherent genetic variation of derived hESC lines. Clearly, this anonymized information would have to be collected for all patients where derivation was attempted, not just the successful outcomes to ensure that no ethical objection could be raised.

One prudent comment was that the standard datasheet must be simple in order to encourage cooperation and ensure data collection. Although we were reluctant to omit any of the sets for data collection given the overwhelming support for each category of the standard, we have attempted to make the description of each completely clear so that there is no uncertainty about exactly what data should be included. The hope is that by making the datasets explicit, the data collection will be simple and reliable, encouraging all groups to contribute and benefit from the sharing of unambiguous, comparable results.

We were greatly appreciative of the many comments received and, where ideas were frequent and common, they were included or discussed. Specific isolated comments were not incorporated into the standard as it was deemed inappropriate when the aim was to report the consensus view from the community. However, when the standard is revisited in Autumn 2008, these points may be included as part of the reassessment process, particularly if the derivation of hESC lines from cleavage-stage embryos

gains momentum [12]. Where comments indicated the need for clarity in some of the data categories, we have altered the wording.

“Clearly, in the field of human embryo work where embryos are in limited supply and every embryo used is extremely valuable, every effort has to be made to extract as much information about those procedures that are carried out as possible.”

– Peter Andrews, Co-Director of the Centre for Stem Cell Biology, Sheffield University, UK

Future steps

It is imperative that this standard evolves with the field and remains relevant and up-to-date. In Autumn 2008 when the next community consultation is held, in order to fine tune the data analysis, instead of a simple three-category response, the Delphi method will be employed to offer robust interpretation of the results [22]. The Delphi method is a systematic interactive forecasting method based on independent inputs of selected experts. The basic Delphi method begins with a series of first-round questions asked individually of experts on the subject; the experts submit their judgments. The results of the first-round judgments are then tabulated and sent back to the experts for modification. These steps have already been completed in this consensus exercise. Each will be asked for the importance of the datasets to be scored using a detailed linear ranking system following the publication of the centrally analyzed results. In essence, the experts are asked in the second round to re-evaluate their original judgments in light of the average estimates calculated in the first round. This procedure of re-evaluation is continued for several rounds until a fairly high degree of consensus is reached, or until the experts no longer modify their previous estimates.

“We think that information on the quality of embryos used for hESC derivation should be part of overall cell line characterization.”

– Alan Trounson, Professor for Stem Cell Sciences, Monash University, and Anna Michalska, Monash University, Australia

The next step is to collect data from participating groups for central analysis. A suggested data collection form designed for clarity and ease of use can be seen in the open-access supplementary information. In addition, the form will be available for download from the UK Stem Cell Bank and *Regenerative Medicine*

journal websites (http://www.futuremedicine.com/doi/suppl/10.2217/17460751.2.4.349/suppl_file/ICSS.pdf). For simplicity, these forms are designed to be either submitted electronically or filled in by hand and returned by fax for analysis. The form contains all the datasets from the consultation as well as a free text response box for comments and suggestions.

Conclusion

The response to the production of a MI convention for the reporting on the derivation of hESC lines has been extremely encouraging. The large number of respondents and their detailed and constructive comments clearly indicate that by far the majority of research groups in stem cell derivation and culture consider standardization an urgent issue. Comments and suggestions from the community have been carefully noted and incorporated into this paper.

In order to facilitate the uptake of the MI convention and initiate the collection of standardized data, the original draft proposal, this consensus paper and the data collection forms are all available free of charge online. Furthermore, laminated paper copies will be circulated both by post and also made available at a number of major international conferences, including the International Society for Stem Cell Research 5th Annual Meeting in Cairns, Australia.

In Spring 2008, we will be contacting the derivation community requesting that they share their data in order to produce the first multi-author review of hESC derivation. Indeed, 85% of those groups questioned have already expressed a willingness to be involved in that publication. We anticipate that this will be the start of a long series of such reviews. We therefore urge every laboratory that is deriving hESC lines to use this consensus MI convention document in order to both build an international view of derivation methodologies and to maximize the value of the subsequent lines for researchers and clinicians. Providing high-quality standardized lines and complete derivation data will greatly enhance the pace of stem cell science. In order to keep the MI convention up-to-date with progress in the hESC derivation field, the authors plan to revisit the standard on a regular basis. The first review is planned for Autumn 2008 when, once again, the entire community will be asked to contribute to the revision. However, if there are comments and suggestions that arise in the meantime, please

forward them directly. It is only by working together to a common standard that the field can move forward and thus help unlock the true potential of stem cells for patients, healthcare providers and the wider society.

“...the grading system and minimum information you proposed are extremely important. The variations in stage and quality of embryos and genetic background of donors used to derive new hESC lines greatly contribute to differences in growth and differentiation patterns among the lines. In addition, establishing and expanding newly-derived cell lines is a complex in vitro process, and the standardization of protocols for hESC culture, maintenance and characterization is equally important.”

– Wu Ma, Director, Stem Cell Center, ATCC, USA

The development of standards must be performed by the scientists but, in addition, scientific journals, funding bodies, regulators and initiatives have a role in supporting the acceptance and necessary progress of standardization, ensuring that the systems in place develop with the field and are kept up-to-date with progress and the changing needs of the community.

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