

ISSUES IN MEDICINE

Stem cell transplantation – a collegial conversation

S H Kidson; reply from G Jordaan

To assist our readers in their understanding of ‘Pioneer’ Paarl neuro sets alarm bells ringing’ in *Izindaba* (p. 8-9), the *SAMJ* sought the opinions of Professor Susan Kidson (SK), one of SA’s foremost stem cell scientists. Based on her insights into the stem cell transplant undertaken on Mr Tommie Prins at the hands of neurosurgeon Dr Adriaan Liebenberg, she offered the following in relation to the stepwise production of suitable stem cells. Dr Gert Jordaan (GJ) was then approached to explain his methodology in raising the stem cells used for the transplant. Dr Jordaan’s reply was received in Afrikaans, an English translation of which is supplied. We believe that the debate will continue...

The Editor

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Steps involved in creating human embryonic stem cells following somatic cell nuclear transfer: Simple summary

SK: Scientists around the globe have uniformly failed to successfully produce such cells from humans, and no one (as far as I can ascertain) has ever tried to introduce such cells into humans. Production of such cells in the mouse and other animal models has been successful.

GJ: Somatiese selkernoordrag (‘SCNT’) is ’n bewese praktyk sedert Dolly se geboorte. Baie reproduksie laboratoriums reg oor die wêreld gebruik hierdie tegniek as ’n standaardprosedure. In die stamsel wetenskap is dit tans die maklikste metode om outologiese pluripotente embrionale stamselle te produseer, en word dit wêreldwyd reeds lankal suksesvol gedoen.

GJ (translation): Somatic cell nuclear transfer (SCNT) has been a proven practice since Dolly’s birth. Many reproduction laboratories worldwide use this technique as a standard procedure. In stem cell science it is currently the easiest method of producing autologous pluripotent embryonic stem cells, and it has been performed successfully on a global basis. **SK:** I agree, somatic cell nuclear transfer *per se* has been carried out for a long time, even prior to cloning of Dolly. I suspect there might be some terminology confusion with respect to using the word pluripotent stem cells: Dr Jordaan seems to refer to the cloned embryo as a pluripotent stem cell, whereas most commonly, this term is specifically used to refer to the cells of the inner cells mass of a blastocyst and cultured, creating an ongoing cell line.

I think it is important that terminology is clarified.

Step 1: Deriving stem cells by somatic cell nuclear transfer

SK: Donor eggs are obtained from willing egg donors and zygotes produced by *in vitro* fertilisation. The zygotic nucleus and mitotic spindle is removed mechanically. There are many variations on how this is done, and it is a very difficult task to carry out. We do not know where the work by GJ is done, how many eggs were obtained, or the details of the patient’s consent regarding the donor eggs.

GJ: Prof. Kidson is verkeerd. Geen sigoot se kern(e) word verwyder nie want daar word geen *in vitro* bevrugting in die proses gebruik nie. Die proses behels bloot die ontkerning (verwydering van die metafase plaat) van ’n oösië d.m.v. mikromanipulasie. Daar bestaan verskeie metodes van manipulasie terwyl in ons laboratorium van

fotodisrupsie (in proses van patentering) en optiese dubbel refraksie gebruik gemaak word.

GJ (translation): Prof. Kidson is wrong. No zygote’s nucleus is removed because no *in vitro* fertilisation is used in the process. The process simply involves denucleation of an oocyte (removal of the metaphase plate) through micro-manipulation. There are various methods of manipulation, and in our laboratory we use photo disruption (in the process of being patented) and optic double refraction.

SK: Yes I understand. The egg spindle and metaphase plate can indeed be removed by micro-manipulations. This is a commonly used alternative procedure for denucleation of the oocyte. Dr Jordaan then goes on to talk about photodisruption and optic double diffraction, but does not explain what these are, and when, how and why these are used. I am unclear as to why such techniques are used if the nucleus and chromosomes have already been removed at metaphase plate stage. Further explanation would be helpful and it would be useful if Dr Jordaan would explain exactly what techniques were used to check that the chromosome removal was 100% successful.

Step 2: Somatic cells (e.g. skin fibroblasts) are obtained (from the patient) and cultured

SK: This is a relatively easy procedure and could well have been achieved.

GJ: Om selkulture (fibroblast) te kweek is basies, maar die stadium van die donor selsiklus, die hoeveelste ‘passage’, die medium behandeling ens., is krities vir suksesvolle embriogenese.

GJ (translation): To perform a cell culture (fibroblast) is basic, but the stage of the donor cell cycle, the ‘passage’, the treatment medium, etc., are critical for successful embryogenesis.

SK: I agree these are important.

GJ: In ons laboratorium is gebruik gemaak van morula blastomere, weens hul ontwikkelingsplastisiteit, as donor karioplast. **GJ (translation):** In our laboratory we used morula blastomeres, because of their differentiating plasticity, as donor karyoplast.

SK: Blastomeres from morulas are indeed pluripotent. But what is not clear here is what is intended to be explained. Is Dr Jordaan saying that he took individual blastomeres from morulas after SCNT, dissociated the cells into individual blastomeres, and transplanted these directly into the patient? I would worry in particular about this, because of the plasticity of such blastomeres, they have the ability to

form both placental and embryonic tissues, and may readily form ectopic tissues or teratomas.

Step 3: Somatic cell nucleus is injected into the enucleated egg and the cell is activated to begin to divide by mitosis

SK: This procedure is technically extremely difficult, and is made up of about 10 - 20 additional steps, with quality assurance protocols (sophisticated molecular, cellular and imaging procedures) carried out at every step. Crucially, the experiment must ensure that all the zygotic DNA and the mitotic spindle is removed from the zygote.

No information has been provided on which steps were taken, and how this was achieved.

GJ: Prof. Kidson is verkeerd. Die donor sel word nie in die ontkernde oösië ingespuet nie maar word wel in die perivitellien spasie geplaas waarna dit na elektroporering met die oösië versmelt.

GJ (translation): Prof. Kidson is wrong. The donor cell is not injected into the denucleated oocyte, but is placed in the perivitelline space, whereafter it is combined with the oocyte after electroporation.

SK: Dr Jordaan explains that the technique he used was the one where the somatic cells are placed below the vitelline membrane, and it is stimulated to fuse with the denucleated oocyte. This is indeed a standard alternative method used to introduce the somatic nucleus into an enucleated egg.

GJ: Die kunsmatige aktivering waaraan die donor-oösië-kompleks blootgestel word is 'n kritieke fase waartydens die epigenetiese aksies (fosforilasie, metylasie, deaktivering van die X-chromosoom ens.) voltooi moet word. Die detail van hierdie proses bly die intellektuele eiendom van ons maatskappy.

GJ (translation): The artificial activation to which the donor-oocyte complex is exposed is a critical phase during which epigenetic actions (phosphorylation, methylation, deactivation of the X-chromosome, etc.) must be completed. The detail of this process remains the intellectual property of our organisation.

SK: I agree. There are many different protocols that have been tested worldwide to optimise this process. SK: Dr Jordaan states that the process he used is the intellectual property of their organisation. I believe in the interests of transparency, this information should be provided or published, particularly if the method he uses is more successful than current ones.

Step 4: The embryo is cultured *in vitro* until a blastocyst is formed (taking about 5 - 7 days)

SK: It is at this stage made up of an outer layer of cells and an inner cluster: these inner cells are the embryonic stem cells. Then many more manipulations must take place to derive stem cells from such a blastocyst. Most commonly, the inner cell mass of the blastocyst is mechanically removed and plated onto feeder cells (usually mitotically inactivated mouse fibroblasts), and fed with medium and growth factors until colonies form. Such pluripotent cells can then be cultured until enough are obtained for use.

It seems likely that cells of unknown genotype and phenotype were produced, but we have not been provided with any technical information, so we cannot say what they are. We do not know if feeder cells were used, and whether they are of human or animal origin.

GJ: Die tegniek wat Prof. Kidson bespreek waar muis 'feeder layers' gebruik word om die binneste selmassa van 'n blastosist op uit te plaas is hoogs riskant by enige mens stamsel werk. Spesiale 'serum-en xeno-vry' medium, wat gebuffer is en alle metaboliete, cytokines en groeifaktore bevat om normale proliferasie te onderhou, is gebruik en die selle is dan op 'n neutrale matriks uitgeplaas.

GJ (translation): The technique that Prof. Kidson discusses, namely where feeder layers are used to plate the inner cells of a blastocyst, is very risky in any human stem cell work. Special 'serum- and xeno-free' medium, which is buffered and which contains all metabolites, cytokines and growth factors needed to sustain normal proliferation, is used and the cells are then placed onto a neutral matrix.

SK: Agree.

GJ: Hierdie mediums word by baie stamsel laboratoriums gebruik, want dit laat die selle fenotopies homogeen en kariotopies normaal.

GJ (translation): These mediums are used in many stem cell laboratories, because it leaves the cells phenotypically homogenous and karyotypically normal.

SK: It would be necessary to provide the evidence of the normal karyotypes and homogeneous nature of the cell culture.

GJ: Dit vertoon hoë vlakke van verskeie antigene wat pluripotensie aantoon sowel as die noodsaaklike gene *Oct 4*, *Nanog* en *SOX2*.

GJ (translation): It shows high levels of various antigens that denote pluripotency as well as the necessary genes *Oct 4*, *Nanog* and *SOX2*.

SK: This sentence does not make sense to me, if the 'it' he is referring to the culture medium. If however, the 'it' refers to the phenotypic and epigenetic state of the derived morula blastomeres, then the sentence would make sense. However, I would ask that this information be provided for scrutiny, in the interests of the medical community and the patient's health.

GJ: Baie aanpassings is in ons laboratorium gemaak t.o.v. die stamsel stadium ten einde maksimum vlakke van die transkripsie merkers en differensiasie proteïene te gebruik om d.m.v. parakriene konneksie, *in vivo*, seldifferensiasie te verkry. Verskeie *in vitro* proewe en baie soortgelyke publikasies het ons hipotese ondersteun.

GJ (translation): Many adaptations have been made in our laboratory with regard to stem cell stage in order to use maximum levels of transcription markers and differentiating proteins to obtain cell differentiation *in vivo* by means of paracrine connection. Various *in vitro* trials and many similar publications have supported our hypothesis.

Step 5: Prior to implantation...

SK: The feeder cells (usually mouse cells, or human skin fibroblasts) must be removed before cells can be injected safely into a patient.

In addition, if such cells were to be used therapeutically, one would need to carry out a sorting process to remove all the cells that have not differentiated along the line of interest.

No information is provided on any of these steps.

GJ: Tydens ons prosedure is glad nie gebruik gemaak van enige muis of mens 'feeder layer' nie wat moontlike verkeerde differensiasie of vreemde selle kan bevat nie.

GJ translation: During our procedure we did not use any mouse or human feeder layers that could possibly contain incorrect differentiation or foreign cells.

SK: Good!

GJ: Baie aandag is geskenk aan die moontlike teratoom vorming na inplanting in die koord self. Daarom was die tipe stamsel van kardinale belang sodat geen anafalaktiese reaksie, weens allogenetiese oorsprong, sou voorkom nie.

GJ translation: Much attention was given to possible teratoma formation after implantation of the cord itself. Therefore the type of stem cell was of cardinal importance, so that no anaphylactic reaction of allogeneic origin should occur.

SK: Herein lies the critical detail that requires full reporting. Given that this is a clinical trial, it is in the interests of all to share what tests were carried out, using what antibody markers (confirming allogeneity). Very importantly, was the potential for

teratoma formation tested? Sharing this information can only give weight and credence to the investigators, and will not detract from the work they have done.

GJ: Voorsorg is getref by die plasing van die stamselle tydens die chirurgiese prosedure, dat slegs fisiese kontak met glial (oligodendrosiete ens.) en neurale selle onder die pia mater gemaak word.

GJ translation: Care was taken with the placing of stem cells during the surgical procedure that only physical contact was made with glial (oligodendrocytes, etc.) and neural cells beneath the pia mater.

SK: This requires much more information, it is not really clear where the cells were placed. In other Phase I clinical trials where stem cells from a variety of sources are placed into injured spinal cords, the cells had already been stimulated to progress along the line of neural or glial differentiation. Drs Jordaan and Liebenberg make no statement as to how the cells that they have implanted will be stimulated to differentiation along a neural lineage.

Moreover, in the two FDA-approved Phase I clinical trials using human embryonic stem cells for treatment of Stargardt's macular dystrophy and the spinal cord injury, human embryonic stem cells were differentiated into retinal pigment epithelial cells and oligodendrocytes respectively prior to implantation. (Note that the trials for spinal cord injury were stopped after patients were injected, for unknown reasons).

There is no indication that in this case, the cells were induced to form oligodendrocytes or neurons prior to injection.

As noted in a recent paper from what is probably the leading group in the world (Egli *et al.*, *Nat Commun* 2011 Oct 4;2:488, which is the group of Douglas Melton and Kevin Eggan of the Harvard Stem Cell Institute), despite many attempts at carry deriving stem cells from human nuclear transfer, all efforts have failed to produce stem cell lines. The only stem cell line purportedly derived by human nuclear transfer has subsequently been shown to originate by parthenogenesis than by reprogramming from the somatic nucleus.

It is highly unlikely that the cells produced by Dr Jordaan are derived from the cells of the patient, and therefore the injected cells

would not be immune-compatible. However, we cannot say for sure because no information has been provided.

GJ: Prof. Kidson se ongelooft t.o.v. die gebruik van die pasiënt se eie DNS is 'n opsetlike ontkenning van die feite en 'n doelbewuste verdagmaking van die proses.

GJ translation: Prof. Kidson's disbelief regarding the use of the patient's own DNA is a deliberate denial of the facts and a deliberate casting of suspicion on the procedure.

SK: I now do have sufficient information to understand that SCNT took place, with nuclei (i.e. DNA) from the patient's fibroblasts, and morulas were produced by cleavage and culture in serum-free defined medium. The morula blastomeres were injected into the patient. Since science works on the basis of critically reviewed evidence, without any hard evidence except media statements, suspicion will continue to be cast on the work until more details are provided.

GJ: Hiermee stel ons dit onomwonde dat die pasiënt se embrionale stamselle deur middel van terapeutiese kloning, na verkryging van 'n velbiopsie, verkry is.

GJ translation: We hereby unequivocally state that the patient's embryonic stem cells were obtained via therapeutic cloning after a skin biopsy.

SK: See response above.

Some further questions

SK: Dr Jordaan refers to his 'Organisation'. Can he indicate what organisation this is, who the members are, and whether they have a scientific advisory board or similar structure guiding and vetting the work?

Dr Jordaan would do himself a service if he were to hold a media briefing or a question-and-answer session with experts, and bring some of the documented evidence of the steps taken to quality assure. He would also be advised to produce copies of all the ethics approvals that have been obtained (from egg donation, patient fibroblast donation, and of course the transplant itself), as here again, if approvals were obtained, there would be nothing to shy away from.