Australian Government
Department of Health
Therapeutic Goods Administration

# Australian Public Assessment Report for Remestemcel-L, *ex vivo* adult human mesenchymal stem cells

Proprietary Product Name: Prochymal

Sponsor: Delpharm Consultants Pty Limited

**March 2015**

TGA Health Safety Regulation

# About the Therapeutic Goods Administration (TGA)

- The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health and is responsible for regulating medicines and medical devices.
- The TGA administers the *Therapeutic Goods Act 1989* (the Act), applying a risk management approach designed to ensure therapeutic goods supplied in Australia meet acceptable standards of quality, safety and efficacy (performance), when necessary.
- The work of the TGA is based on applying scientific and clinical expertise to decision-making, to ensure that the benefits to consumers outweigh any risks associated with the use of medicines and medical devices.
- The TGA relies on the public, healthcare professionals and industry to report problems with medicines or medical devices. TGA investigates reports received by it to determine any necessary regulatory action.
- To report a problem with a medicine or medical device, please see the information on the TGA website <https://www.tga.gov.au>.

# About AusPARs

- An Australian Public Assessment Record (AusPAR) provides information about the evaluation of a prescription medicine and the considerations that led the TGA to approve or not approve a prescription medicine submission.
- AusPARs are prepared and published by the TGA.
- An AusPAR is prepared for submissions that relate to new chemical entities, generic medicines, major variations, and extensions of indications.
- An AusPAR is a static document, in that it will provide information that relates to a submission at a particular point in time.
- A new AusPAR will be developed to reflect changes to indications and/or major variations to a prescription medicine subject to evaluation by the TGA.

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# List of the most common abbreviations used in this AusPAR

| Abbreviation | Meaning |
|---|---|
| aGvHD | acute Graft versus Host Disease |
| BMA | Bone Marrow Aspirate |
| BMMNCs | Bone Marrow Mononuclear cells |
| bMSCs | baboon MSCs |
| BSA | Bovine Serum Albumin |
| cMSCs | canine MSCs |
| CR | complete response |
| DCB | Donor Cell Bank |
| DCs | Dendritic Cells |
| DLI | donor leukocyte infusions |
| DMEM | Dulbecco's Modified Eagle Medium |
| DMSO | dimethyl sulfoxide |
| DP | Drug Product |
| DS | Drug Substance |
| ECG | electrocardiogram |
| ELISA | Enzyme Linked Immuno Sorbent Assay |
| FBS | Foetal Bovine Serum |
| GFP | Green Fluorescent Protein |
| GvHD | Graft versus Host Disease |
| HCT | haematopoietic cell transplantation |
| HLA | human leukocyte antigen |
| hMSCs | human mesenchymal stem cells |
| hPBMCs | human peripheral blood mononuclear cells |
| HAS | human serum albumin |

| Abbreviation | Meaning |
|---|---|
| HSCT | haematopoietic stem cell transplantation |
| IA | intra artery |
| IDO | indoleamine 2,3 dioxygenase |
| IFN-γ | interferon gamma |
| IM | intramuscular |
| IP | intraperitoneal |
| ISCT | International Society for Cellular Therapies |
| ITT | Intention To Treat |
| IV | intravenous |
| KGF | keratinocyte growth factor |
| KLH | Keyhole Limpet Hemocyanin |
| mcMSCs | macaque MSCs |
| mITT | modified Intention To Treat |
| MLRs | Mixed Lymphocyte Reactions |
| mMSCcm | mMSC conditioned media |
| MSCs | mesenchymal stem cells |
| NCP | New Cytomate Product |
| Neor | neomycin resistance gene |
| NK | Natural Killer |
| NO | Nitric Oxide |
| NOAEL | No Observed Adverse Effect Level |
| OR | overall response |
| PBLs | Peripheral Blood Leukocytes |
| PCR | Polymerase Chain Reaction |
| PD | pharmacodynamics |

| Abbreviation | Meaning |
|---|---|
| PHA | phytohemagglutinin |
| PK | pharmacokinetics |
| PP | Per Protocol |
| PR | partial response |
| rMSCs | rat MSCs |
| SAE | serious adverse event |
| SC | Subcutaneous |
| SCP | Standard Centrifuged Product |
| sMSCs | swine MSCs |
| SOC | System Organ Class |
| TBI | total body irradiated |
| TEAE | treatment emergent adverse event |
| TNF-α | tumour necrosis factor-α |
| TSE | Transmissible Spongiform Encephalopathy |
| UEDs | Unanticipated Early Deaths |
| VEGF | vascular endothelial growth factor |

# I. Introduction to product submission

## Submission details

| | |
|---|---|
| *Type of submission* | New Biological Entity |
| *Decision*: | Withdrawn |
| *Date of decision:* | 18 November 2012 |
| *Active ingredient:* | remestemcel-L, *ex vivo* adult human mesenchymal stem cells |
| *Product name:* | Prochymal |
| *Sponsor's name and address:* | Delpharm Consultants Pty Limited<br>5 Alexander Street<br>Crows Nest NSW 2065 |
| *Dose form:* | Cell suspension |
| *Strength:* | 100 x $10^6$ human mesenchymal stem cells per 15 mL bag |
| *Containers:* | Bag/Pouch |
| *Pack size:* | 15 mL |
| *Route of administration:* | Intravenous |
| *Dosage:* | 2 x $10^6$ human mesenchymal stem cells/kg body weight, 2 times per week, for 4 consecutive weeks |

## Product background

This AusPAR describes an application by the sponsor, Delpharm Consultants Pty Limited, to register a new active substance (new biological entity) with an Australian Approved Biological Name (ABN) of remestemcel-L. The proposed trade name is Prochymal. Delpharm Consultants Pty Limited is acting as the Australian sponsor of Prochymal on behalf of Osiris Therapeutics Inc. of Baltimore MD, USA.

This submission is of a novel cellular therapy based medicinal product and is the first of its kind proposed for marketing in Australia. Prochymal is a formulation of *ex vivo* cultured adult human mesenchymal stem cells (hMSCs) intended for intravenous (IV) infusion. The initially proposed indication was as follows:

> *For the rescue of patients ≥ 6 months of age with acute Graft versus Host Disease (aGvHD), refractory to treatment with systemic corticosteroid therapy or other immunosuppressive agents.*

Following adverse recommendation in the clinical evaluation report, the sponsor proposed the following modified indication:

> *Prochymal is indicated in the management of acute Graft versus Host Disease (aGvHD) in paediatric patients. Acute GvHD should be refractory to treatment with systemic corticosteroid therapy and/or other immunosuppressive agents. Prochymal*

*may be used for Grades C and D of the disease in any organ. Prochymal may also be used in the management of Grade B aGvHD involving any visceral organ.*

The proposed Product Information (PI) states that:

*'Prochymal's activity against aGvHD is due to the immunomodulatory properties of hMSCs. Prochymal counteracts T cell mediated inflammatory processes by downregulating the production of the pro inflammatory cytokines, tumour necrosis factor-α (TNF-α) and interferon gamma (IFN-γ). When cell surface receptors of the hMSC are bound by TNF-α, the cells produce prostaglandin E2 (PGE2), which blocks T cell release of TNF-α. The binding of IFN-γ by hMSCs activates secretion of indoleamine 2,3 dioxygenase (IDO). T cell proliferation is thereby downregulated and interleukin-2 expression decreased. Data also suggest hMSCs further reduce inflammation by upregulating the production of anti inflammatory cytokines, interleukin-4 and interleukin-10.'*

The proposed dose is 2 x $10^6$ (two million) hMSCs/kg body weight twice per week for 4 consecutive weeks by IV infusion. The infusion is administered at a rate of 4-6 mL/min in patients with body weight ≥ 35kg. In patients with body weight < 35kg, Prochymal is infused over 60 minutes. The infusions should be at least 3 days apart. There are 100 x $10^6$ hMSCs in each frozen product bag.

It is proposed that if response at 4 weeks is unsatisfactory (partial or mixed response), treatment be continued with the same dose (2 x $10^6$ hMSC/kg) at once a week intervals for a further duration of 4 weeks. If GvHD recurs (any Grade) after 8 weeks treatment, a second treatment cycle is proposed.

No specific guidance is proposed for early discontinuation in case of No Response, but implies that non responders (stable or worsening) should not be treated beyond 4 weeks.

**Acute graft versus host disease (aGvHD)**

Acute graft versus host disease (aGvHD) is an iatrogenic, progressive and lethal complication of haematopoietic stem cell transplantation (HSCT) and donor leukocyte infusions (DLI). In aGvHD, donor T cells recognise non self antigens on the recipient tissues and interact with these antigens resulting in inflammation and host tissue destruction. The postulated pathophysiology of aGvHD is based on a sequential three phase model:

1. injury to the host environment resulting in release of cytokines ('cytokine storm')
2. donor T cell activation, proliferation, and differentiation and
3. organ cell damage caused by direct cytotoxicity or through the production of inflammatory cytokines.[1]

Clinical GvHD has acute and chronic forms. Traditionally, the acute form has been distinguished from the chronic form by the time of onset after haematopoietic cell transplantation (HCT), that is, aGvHD occurring within 100 days, and chronic GvHD occurring beyond 100 days even if the manifestations of chronic disease could not be distinguished from acute disease.[2] However, current consensus opinion is that acute and chronic GvHD should be distinguished by clinical characteristics rather than time after

[1] Ferrara JLM, et al. (1999) Pathophysiologic mechanisms of acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 5: 347-356; Teshima T, Ferrara JLM. (2002) Understanding the alloresponse: new approaches to graft-versus-host disease prevention. *Semin Hematol.* 39: 15-22; DeVetten MP, Vose JM. (2004) Graft-versus-host disease: how to translate new insights into new therapeutic strategies. *Biol Blood Marrow Transplant.* 10: 815-825.

[2] Deeg HJ. (2007) How I treat refractory acute GVHD. *Blood* 109: 4119-4126; Vigorito AC, et al. (2009) Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood* 114: 702-708.

transplantation.[3] Classic aGvHD is characterised by clinical features relating to the skin (maculopapular rash), gastrointestinal tract (predominantly diarrhoea) and liver (cholestatic jaundice), and occurs within 100 days of HCT or DLI.[4]

In a review of therapy in 740 patients with aGvHD, Martin and colleagues[5] found that at the beginning of treatment 81% had rash, 54% had gastrointestinal involvement, and 50% had liver dysfunction. In addition to classic aGvHD, the broad category of aGvHD also now includes persistent, recurrent or late onset disease occurring more than 100 days after HCT or DLI.[6] In contrast to aGvHD, chronic GvHD has more diverse clinical features and can often resemble autoimmune syndromes.[7] The clinical features of aGvHD in children are similar to those in adults, and there appear to be no significant differences between paediatric and adult patients in the clinical symptoms and the pathophysiology of the disease.[8] Data from a recent meta analysis of steroid refractory aGvHD in adults and children treated with MSCs suggests that children might respond better to treatment than adults.[9]

There are no approved treatments for aGvHD, and survival in patients with aGvHD depends on the severity of the disease.[10] While there is no approved treatment for aGvHD, corticosteroids are generally used as first line therapy; if patients do not adequately respond to these agents, mortality is high.[11] Patients with the most severe forms of aGvHD not responding to corticosteroid therapy have expected one year survival rates of 5% to 30%.[12] In patients refractory to first line treatment with systemic corticosteroids, immunosuppressive agents are generally employed as second line treatment despite limited evidence of efficacy and the known risks associated with these drugs.

## Regulatory status

Prochymal for IV infusion was designated as an orphan drug by the TGA on 8 November 2010 for:

> *'the treatment of aGvHD in patients who have undergone HSCT'.*

[3] Filipovich AH, et al. (2005) National Institute of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: 1. Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant.* 11: 945-956; Vigorito AC, et al. (2009) Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood* 114: 702-708.
[4] Shlomchik W. (2007) Graft-versus-host disease. *Nature Rev Immunol.* 7: 340-352; Vigorito AC, et al. (2009) Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood* 114: 702-708.
[5] Martin PJ, et al. (1990) A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood* 76: 1464-1472.
[6] Vigorito AC, et al. (2009) Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood* 114: 702-708.
[7] Shlomchik W. (2007) Graft-versus-host disease. *Nature Rev Immunol.* 7: 340-352; Vigorito AC, et al. (2009) Evaluation of NIH consensus criteria for classification of late acute and chronic GVHD. *Blood* 114: 702-708.
[8] Goker H, et al. (2001) Acute graft-vs-host disease: pathobiology and management. *Exp Hematol.* 29: 259-277; MacMillan ML, et al. (2002) Early antithymocyte globulin therapy improves survival in patients with steroid-resistant acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 8: 40-46; Jacobsohn DA, Vogelsang GB. (2007) Acute graft versus host disease. *Orphanet J Rare Dis.* 2: 35.
[9] Wernicke CM, et al. (2011) Mesenchymal stromal cells for treatment of steroid-refractory GvHD: a review of the literature and two pediatric cases. *Int Arc Med.* 4: 27.
[10] Przepiorka D, et al. (1994) Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 15: 825-828.
[11] Wolf D, et al. (2012) Novel treatment concepts for graft-versus-host disease. *Blood* 119: 16-25.
[12] Deeg HJ. (2007) How I treat refractory acute GVHD. *Blood* 109: 4119-4126; Martin PJ, et al. (1990) A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. *Blood* 76: 1464-1472; Przepiorka D, et al. (1994) Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 15: 825-828; Weisdorf D, et al. (1990) Treatment of moderate/severe acute graft-versus-host disease after allogeneic bone marrow transplantation: an analysis of clinical risk features and outcome. *Blood* 75: 1024-1030; MacMillan ML, et al. (2002) Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant.* 8: 387-394.

No previous submissions have been made by the sponsor to register Prochymal in Australia. It appears that there have been no other submissions for registration of medicines for the proposed indication.

At the date of submission, Prochymal was not approved in any overseas countries for the proposed indication. The product was developed and is manufactured in the US. Submissions for registration have not been made in the US or EU. However, applications to register the product had been made to the relevant Canadian (16 June 2010) and New Zealand (7 June 2011) drug regulatory authorities (Table 1)[13]. The submission was withdrawn in Canada

> 'due to logistical issues [and] timeline limitations... without prejudice to refilling'.

Osiris stated that the Canadian authority has

> 'committed to review the re filled application in an expedited manner'.

Osiris confirms that the data set submitted to the TGA is identical to that submitted in Canada and New Zealand. Osiris states that marketing applications for Prochymal have not been rejected in the USA or Canada.

**Table 1: Overseas regulatory status of Prochymal at the date of submission**

| Country | Regulatory Agency and Directorate | Application Type, And Date | Status |
|---|---|---|---|
| Canada | Health Canada Biologics and Genetic Therapies Directorate (BGTD) | New Drug Submission (NDS): 16 June 2010 | Under evaluation; Submission withdrawn due to logistical issues, timeline limitations and without prejudice to refilling. BGTD has committed to review the re-filed application in an expedited manner. |
| New Zealand | MedSafe | New Medicine Application (NMA): 07 June 2011 | Under evaluation |

# II. Quality findings

## Drug substance (active ingredient)

### Structure

The Drug Substance (DS) is a liquid cell suspension (opaque, off white to pale amber). The active component is *ex vivo* cultured hMSCs. The DS is formulated to a total volume of 15 mL per bag with 5% human serum albumin (HSA) (approved for EU use) and 10% dimethyl sulfoxide (DMSO) (USP) in Plasma-Lyte A (USP) medium.

The DS is not manufactured using a human embryo or human embryonic stem cell, other material sourced from a human embryo or human embryonic stem cell.

There are no similar cell therapy products currently in the ARTG (Australian Register of Therapeutic Goods). This submission represents the first of its type for marketing registration in Australia.

[13] Subsequently Marketing Authorisation for Prochymal for the treatment of aGvHD was granted by Health Canada in May 2012 and by New Zealand MedSafe in June 2012.

**Manufacture**

The manufacture of the DS is at a site based in the United States. Osiris Therapeutics Inc. ('Osiris' or 'OTI') has contracted the principal manufacturing site for the production of the DS and Drug Product (DP). Osiris has retained the responsibility for the review and approval of all executed batch records for DS. Osiris is responsible for the issuance of Certificates of Analysis (CoA) and for the subsequent release of DS in the inventory and storage system. Testing and Quality Control are performed at 4 contract testing sites, Osiris Therapeutics Inc. site, and there are also two sites that perform clinical laboratory testing of donor blood samples. *GMP (Good Manufacturing Practice) clearance certifications for all DS manufacturing sites are still outstanding. These must be issued by the OMQ (Office of Manufacturing Quality) before inclusion on the register.*

Prochymal is manufactured under aseptic conditions in a process involving isolation of human donor cells and *ex vivo* cell culture expansion. There are no antibiotics or antimycotics used in production, nor are there specific bioburden reduction steps or terminal sterilisation processes. A functionally closed system for the *ex vivo* culture expansion of hMSCs was designed and implemented to eliminate almost all opportunities for product contamination. The cells are cultured in tissue culture vessels and washed on a functionally closed system cell washer with the flexibility to process small to large volumes of cell products and is also utilised as a fluid transfer device in the Prochymal manufacturing process. In process controls were established during the process, including control of aseptic processing such as tube welding and sealing, control of operating parameters such as BMA (Bone Marrow Aspirate) inspection and processing, seed culture medium bags, incubation, trypsinisation, cryopreservation, and storage, as well as control of DS safety, identity, purity, quality and potency by QC (Quality Control) lot release testing.

No pooling of donor material takes place in the Prochymal manufacturing process.

The starting material for production of DS is human BMA obtained from the iliac crest of qualified pre-screened young healthy adult donors, who are unrelated and unmatched for blood group and human leukocyte antigen (HLA). The donor BMA is collected, then shipped and stored, and processed within 72 h. The nucleated cells are isolated, washed in culture medium using an automated cell washer, diluted, transferred to a culture bag, then transferred to the tissue culture vessels and cultured in DMEM (Dulbecco's Modified Eagle Medium) containing FBS (Foetal Bovine Serum) for 2 passages. The adherent cells (hMSCs) are harvested, sampled for lot release testing, and formulated and filled in bags, then cryopreserved and stored at ≤ -135°C in LN2 (Liquid Nitrogen) freezer quarantine storage until release of the DS. The DS is also known as the Donor Cell Bank (DCB).

The DS manufacturing process has been sufficiently validated. Validations for aseptic processing, BMA storage and shipping, the controlled rate freezer performance, as well as the cell shipping for the DS/DP have been conducted and are acceptable.

The starting material BMA is obtained from donors who are tested against an extensive screening panel for potential adventitious agents. Donor eligibility, screening and testing requirements are acceptable and in accordance with Appendix 4 of the Australian Regulatory Guidelines for Biologicals.[14] The infectious disease test kits were all deemed to be acceptable for intended use.

There is sufficient evidence that all other materials (biological and non-biological materials) used in the DS/DP manufacturing process are appropriately sourced and tested. However, there is information to indicate that US sourced FBS was used in the

[14] Therapeutic Goods Administration, 'Australian Regulatory Guidelines for Biologicals Appendix 4: Guidance on donor selection, testing and minimising infectious disease transmission via therapeutic goods that are human blood and blood components, human tissues and human cellular therapy products', August 2011

manufacture of currently held stock of Prochymal; the US is considered a controlled risk country for TSE (Transmissible Spongiform Encephalopathy) and acceptable for use, although TGA prefer it to be sourced from lower risk countries. For all future batches, FBS sourced from zero risk countries (Australia and New Zealand) is to be used in the manufacture of Prochymal.

### Physical and chemical properties

The DS consists of hMSCs of stromal origin which can be differentiated into osteoblasts, chondrocytes, and adipocytes. Investigations by Osiris are consistent with the work of others who have shown that hMSCs can be expanded *ex vivo* without the loss of their capacity to differentiate into a variety of tissue types. The hMSCs are known to provide trophic support, and modulate innate immune responses by secreting soluble factors. The MoA of Prochymal is claimed to be due to the immunomodulatory, tissue protective and trophic effects of the hMSCs. Various cell signalling and migration events are proposed to contribute towards the overall therapeutic benefit of Prochymal. The *in vitro* data presented from Osiris in house studies and the cited literature would support the immunomodulatory and anti-inflammatory properties of hMSCs, but proof of concept *in vivo* appears to be very limited. For the purposes of the evaluation, the MoA (Mode of Action) was considered to be theoretically plausible. Establishing proof of concept was not considered to be within the scope of the quality evaluation.

The hMSCs are intended to treat GvHD. GvHD is an active immune response of donor T cells against recipient cells and tissues. The hMSCs counteract T cell mediated inflammatory processes by down regulating the production of the pro inflammatory cytokines, TNF-α and IFN-γ. TNF-α is a major inflammatory cytokine that stimulates the immune response and induces tissue damage through apoptotic pathways. When activated through cell surface receptor interactions with TNF-α, hMSCs produce PGE2 which blocks continued release of TNF-α by immune cells. The decrease in TNF-α inhibits further T cell activation and proliferation. Binding of IFN-γ by hMSCs activates secretion of IDO. This results in cleavage of locally available tryptophan and stops T cell proliferation. T cell proliferation is thereby down regulated, levels of cytotoxic TNF-α reduced, and expression of IL-2 receptor (IL-2Rα) and IL-2, which are critical for T cell proliferation, decreases TNF-α. The hMSCs further reduce inflammation and support tissue repair and protection by up regulating the production of anti-inflammatory cytokines, interleukin-4 and interleukin-10, and certain growth factors such as keratinocyte growth factor (KGF) and vascular endothelial growth factor (VEGF). The presence of activated human peripheral blood mononuclear cells (hPBMCs) up regulated KGF, VEGF and PGE2 secretion by hMSCs, indicating the hMSCs are responsive to activated immune cells.

The hMSCs isolated from donated adult human BMA and expanded under *ex vivo* culture conditions have been characterised using the following criteria:

- Adherence to plastic
- Cell surface antigen markers
- Multipotent differentiation potential and
- Lack of expression of MHC Class II molecules (baseline state).

The above criteria for characterising hMSCs characterisation are consistent with the International Society for Cellular Therapies (ISCT) minimum criteria for defining multipotent mesenchymal stromal cells.[15]

[15] Dominici M, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.

The main characterisation of Prochymal phenotype and identity is based on three markers: CD45-, CD105+ and CD166+. Tri lineage differentiation of hMSCs into osteoblasts, chondrocytes and adipocytes was sufficiently demonstrated for Prochymal in the submission.

The main characterisation of Prochymal potency was based on the immunomodulative, anti-inflammatory properties and tissue repair activities of hMSCs. The viability of the Prochymal cell population, the ability of hMSCs in Prochymal to inhibit IL-2Rα expression on hPBMCs and level of Prochymal hMSC TNF R1 expression associated with significant inhibition of hPBMC proliferation were the focus of investigations in the development of the potency assays for QC and stability indicating tests.

In addition, the established MHC profile supports the low host immune reaction against hMSCs. The submission included *in vitro* evidence that hMSCs do not express HLA-DR (MHC II) molecules and express low levels of MHC I molecules on the cell surface. One of the underlying reasons for use of hMSCs without donor recipient HLA matching is their immune privileged marker profile.

**Specifications**

The DS lot release tests include control tests for identity, purity, potency, appearance, sterility, viral safety, endotoxin, mycoplasma and chromosomal aberrations.

Sufficient justification has been supplied to support the setting of the release and stability specifications.

Health Canada's overall recommendations for the addition of flow cytometric analysis of HLA-DR (MHC II) and MHC I expression to the DS specifications for future Prochymal lots were supported in the evaluation. Low level expression of MHC I and complete lack of HLA-DR (MHC II) expression on MSC may be critical in preventing their immune rejection. *Osiris has committed to implement characterisation of MHC I and HLA-DR for the DS lot release testing.*

Osiris has developed a specific assay panel to define potency and phenotype. These assays are used to identify, characterise and control the DS/DP. These are acceptable.

Appropriate validation data have been submitted in support of all test procedures and overall the DS specifications are acceptable.

***Definition of potency***

There are no industry established reference standards for a cell therapy product. Potency of most cell therapies has not been fully characterised. Techniques for doing so are still being developed at the basic research level. There is international consensus that due to the complex mode of action of cellular therapies, multiple complementary assays should be performed to adequately assess product potency. Therefore, Osiris developed three assays to evaluate potency of Prochymal, with the key characteristic of the hMSCs used for the treatment of aGvHD being their suppression of the underlying inflammatory disease:

1. Cell viability: surrogate potency assay
2. TNF RI expression: measurement of TNF RI expression level in hMSC cell lysates. Expression of this receptor on hMSCs has been linked to its anti-inflammatory and immunomodulatory biological activities. The specification acceptance limit for significant inhibition of T cell proliferation and
3. IL-2Rα expression on hPBMCs: bioassay measuring the indirect ability of the hMSCs to inhibit expression of IL-2Rα expression on hPBMCs. The assay method allows the hMSCs undergo some recovery time when placed back in co culture conditions, prior to the determination of IL-2Rα expression on stimulated hPBMCs. This assay is used

as an *in vitro* measure of hMSC functionality. IL-2Rα expression supports growth of activated T cells and is an early indicator of T cell activation. Its inhibited expression leads to suppression of T cell proliferation and is an underlying mechanism for a number of immunosuppressive drugs including cyclosporine, tacrolimus, and rapamycin.

The proposed specifications to control potency properties of the DS/DP are relevant to the dose form and its intended clinical use and the test assays are accepted to be sufficiently validated and robust. However, it is unknown whether the *in vitro* potency measures will have any physiological relevance and this should be determined by the nonclinical and clinical assessors.

The sponsor has acknowledged the challenges of developing potency assays for this type of therapy, but maintains the position that the assays are scientifically valid and the most robust QC test assays available at the current time. In addition, it has been proposed that the panel of testing approach serves to address the limitations associated with each individual test assay. Certainly, the development of the TNF R1 and IL-2Rα potency assays would satisfy the outlining principles in the available international guidelines of relevance to cell therapy products, where the focus is upon defining desirable product performance characteristics. There were no quality issues raised in Health Canada or MedSafe New Zealand's assessment of Prochymal in relation to the suitability of these assays for use in the definition of potency.

### Stability

The real time data submitted from the two associated primary study protocols support a proposed shelf life of 48 months for the DS stored at ≤ -135°C in the liquid nitrogen vapour phase.

Note that a number of final study reports for these protocols have been requested as a proposed condition of registration.

There was an outstanding issue of concern on the adequacy of the TNF R1 potency test employed as a specific quantitative measure of DS preservation/degradation during storage. Advice on this matter was sought from the PSC (Pharmaceutical Subcommittee) of the Advisory Committee on Prescription Medicines (ACPM) at its 147th meeting. The PSC acknowledged the inherent difficulties with potency assays for hMSCs and advised that cell viability assay is the best indicator of product degradation at the current time. The PSC also agreed that the TNF RI test is not an appropriate test for assessing stability of the DS/DP.

## Drug product

### Packaging

The overall packaging consists of an immediate container bag, a secondary packaging aluminium cassette, and a primary packaging carton. The immediate container has been tested for its compatibility with DS and the excipients. An alternative bag has also been approved as the one used in the previous manufacturing runs is no longer available. The sponsor has committed to place on stability the first manufactured product lot that uses the alternative cryogenic freezing bags.

After sealing the immediate container bag, each bag is placed into an aluminium canister/cassette. This secondary packaging container is designed to enclose and protect the freezing container.

### Formulation(s)

The drug product (DP) (Table 2) is packaged in freezing containers in a final volume of approximately 15 mL.

**Table 2: Composition of the DP**

| Name of Ingredient | Function |
|---|---|
| hMSC | Active Agent |
| HSA | Protein that provides cellular stabilization and cell protection from shearing forces |
| DMSO | Cryoprotectant Solution |
| Plasma-Lyte A | Diluent providing physiological osmolality and pH |

All of the clinical lots produced and utilised in the Phase 2/3 clinical trials and expanded access program were comprised of the same DP formulation as that proposed for commercial use.

Preparation of the DP for administration involves thaw and reconstitution with Plasma-Lyte A or equivalent diluent. After thaw, 25 mL of Plasma-Lyte A (or equivalent) is added to the DP unit for a total volume of 40 mL (15 mL of the DP dose unit volume plus 25 mL Plasma-Lyte A). The total volume administered per infusion depends on dosage which is determined from the patient's weight (kg). The dose for clinical use is 2 x $10^6$ MSC/kg. Based on clinical experience in refractory aGvHD, Prochymal should be administered twice weekly for four weeks.

Once reconstituted, Prochymal must be kept at room temperature and must be infused within 5 h from thaw of the product.

### Manufacture

The DP manufacturing process is US site based. In 2003, Osiris contracted manufacture of the DP, conduct final inspection, testing, and storage and distribution of DP. Osiris has retained the responsibility for the review and approval of all executed batch records for DP. Osiris is responsible for the issuance of CoA for subsequent release of product in the inventory and storage system. Testing and QC are performed at 2 contract testing sites and the Osiris Therapeutics Inc. site. GMP clearance certifications for all DP manufacturing sites are outstanding.

The manufacture of the DP consists of the final three passages (thaw and seeding of the DS), Passage 2 (P2) → Passage 3 (P3) → Passage 4 (P4) → Passage 5 (P5), harvest. Following the harvest, the DP is formulated and stored at ≤ -135°C in quarantine pending release. The sponsor has established process control parameters. There are no specific bioburden reduction steps or terminal sterilisation processes for the DP.

Based on the cell yield at harvest, the pooled and washed *ex vivo* cultured adult hMSCs are either concentrated or diluted to the appropriate concentration. The DP formulation is then prepared by the addition of cryoprotectant solution. At final harvest, each DP freezing container (bag) is filled with approximately 125 x $10^6$ viable cells in 15 mL of Plasma-Lyte A solution containing HSA and DMSO.

The DP manufacturing process has been sufficiently validated and the successful execution of validation protocols for the DS and DP would serve as confirmation that the entire manufacturing process of Prochymal can be executed consistently.

However, subsequent to the completion of the process validations for the DP, a number of manufacturing process changes were implemented in 2009. These were not limited to, but include changes to cell culturing parameters. The contract manufacturer completed three full DP engineering runs, using one lot from each of three different donors under non GMP conditions, to provide assurance that the process changes incorporated did not affect the safety or quality of the cells. The supporting data submitted was deemed insufficient to justify the change as it could represent a reduction in process control.

There is an additional concern that due to the insufficient characterisation of the change it was difficult to compare the quality of the DP manufactured under the commercial process compared to the product tested in Phase 3 clinical trials. Advice was sought from the PSC on the adequacy of the process changes for cell culturing parameters at the 147th meeting.

### Specifications

The proposed specifications are relevant to the clinical use of the product. The lot release tests include tests for identity, purity, potency, appearance, post thaw viability, residual BSA (Bovine Serum Albumin) and trypsin, sterility, endotoxins, mycoplasma with established acceptance criteria. Justification of specifications has been supplied to support the setting of the release and stability specifications.

The sponsor was asked to justify why certain tests had been included for the DS specifications but not for the DP specifications. The DS is subjected to a further three passages in culture and it would be more prudent to test for chromosomal aberrations at the later DP stage from a quality and safety perspective to address the known potential for hMSC transformation in *ex vivo* cultures. The sponsor claims the additional processing from DS to DP of 3 passages does not alter the low risk of transformation of stem cells in culture and has not seen any evidence clinically that there is a safety concern due to potential transformation. The sponsor has agreed to institute a monitoring program on DP to provide additional information to satisfy the concerns raised.

The results of batch analyses indicate that the specification limits for phenotypic and potency tests could be tightened and this was raised with the sponsor during the evaluation. However, there were no identifiable trends that would require corrective action. The sponsor has committed to analysing the batch analysis data for the DS and DP and will revise and tighten all applicable lot release specifications as appropriate prior to release of any newly manufactured DS or DP lots.

Overall, the DP specifications are acceptable, and appropriate validation data have been submitted in support of the test procedures.

### Excipients

There is the evidence that the excipients are appropriately controlled according to the intended clinical use.

### Characterisation of impurities

Impurities associated with the manufacture of the DP are stated to be residual BSA, residual heparin, and residual trypsin and particulates inherent to the manufacturing process. Impurities such as viral and other adventitious agents are controlled at the DS manufacturing stage. Testing for residual heparin is not performed because the estimated residual level in the DP is lower than the limit of detection for the most sensitive ELISA

type assay. Heparin is of clinical use and the residual is claimed to have no safety impact on the DP. Residual testing has been implemented to ensure that minimal amounts of those reagents used in the manufacturing process that are of animal origin are present in the final product.

The presence, the dead cells, cell debris, and non hMSCs were not stated as DP impurities and there were concerns raised on this during the evaluation. The product characterisation information was also reviewed in relation to this matter. The potential for unidentified CD45-/CD105-/CD166- cells to be present as low level impurities in the DP was not fully addressed by the sponsor. The evaluation also concluded that low level fibroblastic contamination of the DP (<1%) cannot be ruled out based upon the similarities in fibroblast: MSC properties reported in the literature and the ability of the QC lot release tests to discriminate these. However, sufficient evidence has been provided in the submission that such cell type impurities are controlled to acceptable limits by the manufacturing process and lot release tests.

### Stability

Stability data have been generated under stressed and real time conditions in order to characterise the stability profile of the DP. There was an outstanding issue of concern on the adequacy of the potency tests employed for the determination of Prochymal preservation during storage.

The real time data supplied from the long term DP stability studies support a proposed shelf life of 36 months for the DP stored at ≤ -135°C in the liquid nitrogen vapour phase.

A number of the stability studies are ongoing and the final reports have been requested as a condition of registration.

## Biopharmaceutics

Biopharmaceutic information is primarily available under the Summary heading of 'Physical and Chemical Properties'. Information on biopharmaceutical testing is available under Summary headings for the DS/DP 'Specifications'.

Bioequivalence data was not required for the submission as the sponsor is not claiming bioequivalence to an existing medicine.

Bioavailability data is not required for this type of product. It is expected that biodistribution data concerning this product would be supplied for the nonclinical and/or clinical evaluation. Please refer to the submission or the relevant evaluation reports for further details on biodistribution.

## Advisory committee considerations

The administrative, product usage, chemical, pharmaceutical, microbiological and biopharmaceutic data (as applicable) submitted in support of this application have been evaluated in accordance with the Australian legislation, pharmacopoeial standards and relevant technical guidelines adopted by the TGA (or otherwise).

### Primary evaluation

Numerous matters arising from the Round 1 and 2(a/b) evaluations were raised with the sponsor. The sponsor has sought to address all of the matters raised and proposed additional teleconference meetings to clarify matters associated with the responses to questions. The sponsor has satisfactorily addressed all matters, with the exception of two matters which were taken to the PSC for advice at its 147th meeting. PSC provided

sufficient advice on one matter and requested that further input from the sponsor be sought on the other matter relating to manufacturing process changes for the commercial DP. The sponsor has supplied further input on this matter and a final recommendation on product approval has been made.

*Final report recommendation: Refer to 'Quality summary and conclusions' section below.*

### Container safety evaluation

Matters arising from the Round 1 evaluation were raised with the sponsor. The sponsor has satisfactorily addressed these matters and there are no outstanding issues.

*Final report recommendation: Prochymal is acceptable for registration with respect to container safety.*

### Endotoxin safety evaluation

Matters arising from the Round 1 evaluation were raised with the sponsor. The sponsor has satisfactorily addressed all matters. Although, it was noted that the sponsor had misinterpreted some of the questions and it appeared that the responses on endotoxin testing matters may not have been formulated by the finished product manufacturer actually responsible for bacterial endotoxin testing.

*Final report recommendation: There are no further bacterial endotoxin safety related issues that need delay the approval of this application.*

### Sterility safety

Numerous matters arising from the Round 1, 2 and 3 evaluations were raised with the sponsor. The sponsor has satisfactorily addressed all matters, with the exception of a single matter relating to adequacy of testing on in process wash solutions which was taken to the PSC for advice at its 147th meeting. PSC requested that further input from the sponsor be sought on the matter. Due consideration has been given to further input from the sponsor. The outstanding matter has been satisfactorily resolved.

*Final report recommendation: There are no objections from a microbiological perspective to the approval of this application.*

### Pathogen safety (scope includes viral, prion and mycoplasma safety)

Matters arising from the Round 1 and 2 evaluations were raised with the sponsor. The sponsor has satisfactorily addressed all of the matters.

*Final report recommendation: there are no outstanding issues of concern with respect to pathogen safety.*

### Issues of importance

The PSC provided advice on three outstanding issues of importance at its 147th meeting. Details of the issues can be found in the final evaluation report and the meeting proceedings and papers. The PSC was in agreement with the issues raised and the major advice was to seek further input from the sponsor on two of the issues to seek satisfactory resolution and further assist with ACPM deliberations. The three issues and their resolution are summarised below:

1. **Inadequacy of the sterility testing of the in process wash solutions.** The system used as a rapid sterility test has undergone method suitability testing but has not

undergone method validation to show that the 7 day incubation period used is at least equivalent to the 14 day compendial sterility test.

**Resolution:** As a result of the sterility evaluation dated 3 September 2012 and after the 147th meeting of the PSC, further input was requested and provided by the sponsor in an email communication dated 18 October 2012. The sponsor states that Osiris has already begun the process of moving away from the system. Upon restart of manufacturing, the Wash solution will be prepared by the contract manufacturer and the standard USP/EU 14 day sterility testing will be performed prior to use in manufacturing. The system will no longer be used. *This is acceptable. TGA approval of the manufacturing process is conditional to there being an approved method validation of the USP/EU 14 day sterility test for the in process wash solutions.*

2. **The appropriateness of the TNF R1 potency assay to reliably detect product degradation and loss of functionality has not been demonstrated.**

   **Resolution:** As a result of the primary evaluation dated 5 September 2012, the PSC acknowledged the inherent difficulties with potency assays for hMSCs and the lack of a generally accepted suitable assay for such products. The PSC noted that the TNF-R1 assay uses cell lysates containing the surface expression marker and considered that this is not an appropriate test for assessing cell degradation or stability of the drug product given that it does not discriminate between live and dead cells. The PSC advised that the cell viability assay, which is included in the long term DP stability protocols, is currently the best indicator of product degradation. *The PSC advice has been noted in the final evaluation report and the matter is closed.*

3. Changes introduced in 2009 to streamline the manufacturing process for the commercial DP were not appropriately validated or justified. From a quality perspective, the concern was that impact of the change was not appropriately characterised and process control was not satisfactorily demonstrated. As a result there is potential for the changes to effect clinical performance and safety.

   **Resolution:** PSC were in agreement with that the concerns were valid and process control was not demonstrated. Osiris has proposed to reinstate the following process steps to ensure that the biological characteristics and properties of the DP are consistent:

   a. Microscopic observation of cell culture morphology and growth characteristics throughout processing.

   b. Starting cell count at the beginning of DP production.

In addition, Osiris agrees to develop a protocol to perform additional characterisation of the cells. The protocol will be provided to the TGA for review and comment prior to its execution. *Reinstatement of the process steps outlined above by the sponsor is deemed sufficient to control the current manufacturing process. Additional characterisation, as proposed, would only be required if the sponsor intend to validate the proposed streamlined process in the future.*

## Quality summary and conclusions

The Quality evaluator recommends that there is no objection to the approval of Prochymal (remestemcel-L, *ex vivo* adult human mesenchymal stem cells), cell suspension, 100 x $10^6$ cells per 15 ml bag, on quality grounds. However, the following issues should be noted:

- A number of changes have been introduced to the manufacturing process for Prochymal in response to issues raised by TGA. On this basis the manufacturing process utilised for generation of currently held stock of Prochymal is not in compliance with TGA's requirements and should not be released in Australia.

- The current proposed labels and packaging are not acceptable and must be approved prior to release of product in Australia.
- The current sterility test method used for the in process wash solutions is not acceptable. The proposed new USP/EU 14 day sterility test must be validated and approved by TGA before release of product in Australia.

**Conditions of registration**

The following non-standard conditions of registration should be applied:

a. The product to be released in Australia must have been manufactured in accordance with the approved TGA manufacturing process, which includes:

   i. supporting method validation for the USP/EU 14 day sterility test of the in-process wash solutions and

   ii. the following process steps:

      microscopic observation of cell culture morphology and growth characteristics throughout processing

      starting cell count information at the beginning of DP production

b. The sponsor should ensure that the following stability study reports are provided to the TGA in support of the proposed DS/DP shelf lives:

   i. Final 60 month DS stability report for the completed DS Study, which includes the statistical analysis and determination of the degradation profile, as primary evidence to confirm the DS shelf life.

   ii. Final DS stability report for the ongoing DS Study, which includes the statistical analysis and determination of the degradation profile, as necessary supporting evidence to confirm the DS shelf life. In the interim, the TGA should be notified of any out of specification results or identified trends as these become known. This evidence is considered necessary due to some of the issues which were highlighted in relation to establishing and executing the main DS stability.

   iii. Final 48 month DP stability report for the five commercial lots, which includes statistical analysis and determination of the degradation profile, as primary evidence to confirm the DP shelf life.

   iv. 42 month interim DP stability report for the single lot, which includes statistical analysis and determination of the degradation profile, as supporting evidence to confirm the DP shelf life.

   v. The sponsor is required to provide TGA with stability data performed on the first manufactured product lot using the alternative cryogenic freezing bags.

c. The proposed labelling and packaging on both the primary pack and container (bag/pouch) are not compliant with TGO69. Prior to supply of product in Australia compliant labels must have been approved by TGA.

# III. Nonclinical findings

### Introduction

The sponsor submitted a combination of peer reviewed articles and in house studies for toxicology assessment of Prochymal. Nonclinical data consisted of primary pharmacology

(literature based), safety pharmacology, biodistribution, acute and repeat dose toxicity, and immunotoxicity studies. Prochymal efficacy was studies in animal models of hematopoietic stem cell engraftment after myeloablation and skin grafting, but not in animal models of GvHD, which are of limited utility and do not elicit similar clinical symptoms to the human disease. Safety pharmacology and toxicity studies were not performed with Prochymal, but utilised MHC-mismatched homologous MSCs in rats, dogs, pigs and monkeys, which is relevant to the clinical use of Prochymal (allogeneic unmatched MSCs ) The use of Prochymal in xenogeneic models would however confound interpretation of immune system responses, and thus be limited in its utility. The respiratory safety pharmacology study, and repeat dose toxicity studies were conducted with rat MSCs, using different strains as an allogeneic model. Acute toxicity studies in dogs utilised autologous MSCs. A 6 week tumourigenicity study (non GLP [Good Laboratory Practice]) in nude mice was conducted with a developmental hMSC preparation. Local tolerance was investigated in repeat dose toxicity studies. No genotoxicity or reproductive toxicity studies were submitted, which is acceptable.

The product development initially focussed on improving haematopoietic stem cell regeneration after myeloablation, and early non GLP acute toxicity studies in total body irradiated (TBI) dogs reflected this aim.

Note: In this nonclinical report, 'mesenchymal stem cells' (MSCs) generally refers to the *ex vivo* expanded cells and not Prochymal in its clinical formulation, which was not tested.

## Pharmacology

### Primary pharmacology

The sponsor presented data on immunomodulation, tissue homing, tissue protection and tissue repair properties of the test article in the form of nine peer reviewed articles. The sponsor stated that an acute GvHD model to test efficacy in rats, the species used in the pivotal toxicity studies, was not a representative model of the clinical disease. Thus, 'Prochymal's efficacy for the treatment of refractory GvHD must be determined from clinical data.' The nonclinical data therefore only provides modest information with regard to the clinical dose or dose regimen in relation to efficacy, nor on the suitability of the proposed product potency assays.

#### *Immunomodulation: in vitro studies*

Evidence of *in vitro* immunomodulatory capabilities of Prochymal were offered in the following five peer reviewed publications:

- Bartholomew et al.[16] utilised Mixed Lymphocyte Reactions (MLRs) to assess immune modulation in baboon MSCs (bMSCs). A total of 1.0 x $10^2$ to 1.0 x $10^5$ bMSCs were used to stimulate 1.0 x $10^5$ allogeneic Peripheral Blood Leukocytes (PBLs). Addition of bMSCs resulted in a greater than 50% reduction in proliferative response from Con A stimulated allogeneic PBLs. The suppression was dose dependent and could be partially reversed in the presence of IL-2. The bMSCs suppressed proliferation irrespective of their origin (that is, same source as responder, stimulator or third party).
- Klyushnenkova et al.[17] added purified hMSCs (2.0 x 105/well) to 1.0 x $10^6$ purified human T cells at Day 0 or Day 4 of MLR assays. Muted allogeneic T cell proliferation

[16] Bartholomew A, et al. (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol.* 30: 42-48.
[17] Klyushnenkova E, et al. (2005) T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. *J Biomed Sci.* 12: 47-57.

was observed in the presence of hMSCs. Retroviral based over expression of CD80 and CD86, or IFN-γ induced expression of co stimulatory molecules in MSCs did not induce proliferative T cell response. Trans well cultures using hMSCs (2.0 x $10^5$ cells/well) and purified human T cells (1.0 x $10^6$ cells/well) also suppressed T cell proliferation, indicating secretion of soluble factors by hMSCs which inhibit proliferation. The study further found suppression to be dose responsive and genetically unrestricted. The MSCs were differentiated along osteogenic fates; however, differentiated MSCs did not elicit a proliferative response despite induction of MHC Class II molecules by IFN-γ.

- Aggarwal et al.[18] examined immunomodulatory effects of hMSCs on Dendritic Cells (DCs), Helper T Cells (TH1 and TH2 cells) and Natural Killer (NK) cells. 2.0 x $10^4$ hMSCs were cultured in the following ratios; MSCs:DC1 (1:10), MSCs:DC2 (1:1), MSCs:TH1 (1:10), MSCs:TH2 (1:10), MSCs: TReg (1:10) and MSCs: NK (1:1). Under above co culture conditions, the following changes to cytokine concentrations were respectively observed: approximately 50% reduction in TNF-α, approximately 140% increase in IL-10, approximately 50% reduction in IFN-γ, approximately 500% increase in IL-4, increase in regulatory T cells (T Reg cells), and approximately 80% reduction in IFN-γ. In addition, a greater than 70% increase in PBMC proliferation was observed in the presence of the PGE2 inhibitors, indicating a possible role for PGE2 in MSC mediated immune modulation.
- Di Nicola et al.[19] co cultured autologous or allogeneic hMSCs (5.0 x $10^4$ cells/well) with activated T lymphocytes (5.0 x $10^4$ cells/well) stimulated by either, allogeneic DCs or PBLs. A 60% to 98% reduction in proliferation was observed when autologous hMSCs were added to MLRs of DC or PBL stimulated T cells. A 65% reduction in proliferation was also observed when hMSCs were co cultured with T lymphocytes stimulated with polyclonal activators, such as PHA (phytohemagglutinin). The authors further demonstrated that suppression of proliferation is likely mediated through TGF-β1 and HGF.
- Tse et al.[20] co cultured IFN-γ pre-treated hMSCs (1.5 x $10^4$ cells/well) with PBMS (1.0 x $10^5$ cells/well) co stimulated with an anti-CD28 antibody. The hMSCs did not induce a proliferative response when co cultured with PBMCs, and they suppressed proliferation of PBMCs stimulated by third party PBMCs. The hMSCs also suppressed proliferation of T cells stimulated by anti CD3 and CD28 antibodies. By using antibodies and inhibitors against TGF-β1 and PGE2, the authors demonstrated that these molecules were unlikely to mediate suppression of proliferation. These findings were in contrast to those of Di Nicola et al. and Aggarwal et al. who demonstrated, respectively, putative roles for TGF-β1 and PGE2 in inhibition of MSC mediated suppression of T cell proliferation.[21]

Taken together, the above studies indicate a general immunomodulatory capacity for MSCs *in vitro*. However, there is concern regarding the strength of data used for claims of the immunomodulatory capability of MSCs, as indicated below:

- The ratios of MSCs:PBMCs or MSCs:T cells used in these studies ranged from 1:1 to 1:10; and the cells cultured in 96 or 6 well plates. Therefore, large volumes of cells (up to 2.0 x $10^5$ per well of MSC's and 1.0 x $10^6$ per well of T cells) were located in close

[18] Aggarwal S, Pittenger MF. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105: 1815-1822.
[19] Di Nicola M, et al. (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 99: 3838-3843.
[20] Tse WT, et al. (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75: 389-397.
[21] Di Nicola M, et al. (2002) Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 99: 3838-3843; Aggarwal S, Pittenger MF. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105: 1815-1822.

proximity and in small culture volumes. The *in vitro* conditions used for these studies do not appear to appropriately represent *in vivo* conditions where the distance and ratios between stimulator and responder cells are likely to be larger. Given that the above studies suggest MSCs impart their actions in a dose dependent manner, it would have been beneficial to conduct *in vitro* studies under physiologically relevant/comparable conditions. It is however acknowledged, that at the time of performing these studies, the technology available did not allow for adequate approximation physiological conditions.

- The above studies have tested several secreted molecules (TGF-β1, HGF, and PGE2) as most likely candidates for MSC mediated suppression of proliferation. However, the studies reported conflicting outcomes for each candidate molecule. As there was no standardisation between each study in terms of (a) culture conditions, (b) passage numbers, (c) cellular homogeneity of culture or (d) methodology, it is difficult to substantiate the veracity of either claim. Therefore, there is no clear presentation of molecular mechanisms underpinning MSC mediated suppression of T cell proliferation *in vitro*. To this end, the data suggest MSC-mediated immunosuppressive effects are multimodal.
- Some candidate secretory molecules, such as PGE2, demonstrate a bell shaped secretion profile with reduced concentrations after 4-5 days in MLR culture (data not shown).[22] The mechanistic effect of such secretion profiles on effective long term *in vivo* immunomodulation was not explored or addressed.
- Data on the immunomodulatory capabilities of differentiated MSCs was limited. Since differentiated *in vitro* cultures are impure and thus may contain undifferentiated MSCs, it is not possible to draw conclusions on the immunomodulation capacity of MSC progeny *in vitro* or *in vivo*. Furthermore, the data was restricted to osteogenic differentiation; no investigations were conducted on immunomodulatory capacity of MSCs differentiated towards the GI (gastrointestinal), skin or hepatic lineages, which appear to be most affected by aGvHD.
- Tse et al.[23] demonstrated that MSC conditioned media could suppress proliferation of T lymphocytes. This would suggest that if physical presence of MSCs is not essential in vitro, infusion of MSCs may not be required to elicit a similar immunomodulatory response *in vivo*. This possibility was however not explored and warrants further investigation.

### *Immunomodulation: In vivo studies*

No animal models were provided to interrogate mechanisms of immunomodulation in detail, *in vivo*. The study using baboons by Bartholomew et al.[24] showed a brief increase in graft survival time when bMSCs were administered prior to initiation of donor or third party skin grafts, demonstrating immunemodulatory effects in vivo; however, the study was not in the context of immunomodulation in total body irradiation or GvHD models.

### *Tissue homing: in vitro studies*

No *in vitro* experimental data were provided to complement the *in vivo* tissue homing studies. Given the concerns highlighted in 'Tissue homing: *In vivo* studies', *in vitro* modelling using specific cytokines to which MSCs respond would have been beneficial.

[22] Aggarwal S, Pittenger MF. (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105: 1815-1822.
[23] Tse WT, et al. (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75: 389-397.
[24] Bartholomew A, et al. (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol.* 30: 42-48.

### *Tissue homing: in vivo studies*

The 'homing' capabilities of MSCs *in vivo* were investigated using two non human primate models.

- Chapel et al.[25] exposed 12 macaques to a combination of different bone marrow mononuclear cells (BMMNCs) and macaque MSCs (mcMSCs) doses and different TBI conditioning regimes. The mcMSCs were administered IV, a proportion of which were labelled with Green Fluorescent Protein (GFP). Tissue samples were analysed using a genomic Polymerase Chain Reaction (PCR) based method. Varying copy numbers of GFP were demonstrated in haematopoietic tissues (bone, bone marrow, and spleen), skin, skeletal muscle, tendons, stomach and gut up to 82 days post infusion. No GFP was detected in brain, liver, kidney, lung, thymus, rectum and testicles at necropsy.
  - Differences in GFP copy numbers were observed in different skin and skeletal muscle tissue corresponding to locations exposed to different levels of radiation damage. However, no statistically significant correlations could be established due to small sample numbers.
- Devine et al.[26] infused three juvenile baboons, of which two were conditioned with 1000cGy radiation, with either GFP labelled autologous MSCs or GFP labelled allogeneic MSCs. Both irradiated test subjects showed similar tissue distribution. The non-irradiated test subject also demonstrated presence of bMSCs, however at lower copy numbers.
  - The GI tract had the highest concentration of GFP copies of any test subject. Due to small sample size however, it was not possible to determine the statistical significance of the difference in GFP signal distribution. Presence of GFP was also detected in kidney, thymus, lung and liver in varying copy numbers between the three subjects. The presence of GFP was detected up to 21 months following infusion.

The above studies were provided to demonstrate homing capabilities of primate MSCs to damaged tissue sites in general. However, the findings must be considered preliminary for reasons discussed below:

- Of the two studies, only Devine et al.[27] provided an appropriate negative control (non-irradiated, MSC infused) to determine the true 'homing' capability of MSCs in irradiated versus non irradiated conditioning. However, as there was only one subject utilised for each condition, it was also not possible to quantitatively or semi quantitatively assess the homing capabilities of the test article. The non-irradiated control in Chapel et al.[28] in contrast was not infused with MSCs, therefore, precluding a comparison between the non-radiated and radiated animals.
- The PCR based method of detection did not allow for clear cellular resolution within the host tissues. It is therefore not possible to ascertain if the cells positive for GFP are closely associated with regions of tissue damaged caused by irradiation, as hypothesised.
- The PCR based detection method also does not allow identification of the GFP positive cells as MSCs *in vivo*. MSCs require very specific and defined conditions for

[25] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.
[26] Devine SM, et al. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 101: 2999-3001.
[27] Devine SM, et al. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 101: 2999-3001.
[28] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.

maintenance of naive stem like state both *in vivo* and *in vitro*. Given the length of survival time in vivo (up to 21 months, Devine et al.[29]) it is unlikely all cells were able to maintain their original 'stem like' state in the host tissue, unless grafted to specific niche tissue, such as bone marrow. In the absence of cell type identity, it is difficult to validate the proposed mechanisms of action of the test article *in vivo*.

- The authors of Chapel et al.[30] note that populations of MSCs used for transplantation are heterogeneous in nature, as determined by expression of SH2 and SH3, which were used as markers of MSCs in this study. However, there was no determination on which SH2/SH3 positive cells successfully 'homed' to the target tissue or any effects of heterogeneity in MSC population *in vitro* on the efficacy of treatment.
- Chapel et al.[31] also proposed that MSCs might 'home' to regenerating tissue as opposed to severely damaged tissue; this is mechanistically different to the homing method proposed by the sponsor. No attempt was made to reconcile the underlying differences of the homing mechanisms.

### *Tissue protection*

A peer reviewed publication by Ellison et al.[32] was provided to illustrate tissue protection capabilities of KGF (FGF-7) in the C56BL/6 → (C56BL/6 x DBA/2)F1 hybrid mouse model of GvHD. The study demonstrated that 5 µg/kg daily injections of KGF prevented development of GvHD, endotoxemia and GvHD induced death mediated through Nitric Oxide (NO) and TNF-α in intestinal tissue.

While this study demonstrated the *in vivo* tissue protection potential of KGF, extrapolation to MSC secreted KGF mediated tissue protection in aGvHD is difficult for reasons outlined below:

- The study itself did not utilise MSCs to deliver KGF to damaged tissue or demonstrate expression of KGF by MSCs. Data was not presented on factors which induce and regulate KGF expression in MSCs.
- No data was presented on how much KGF is produced by a given number of hMSCs. It is difficult to extrapolate the rodent response to 5 µg/kg daily injections to hMSC secreted KGF *in vivo* in treatment of GvHD in humans.

### *Tissue repair*

Two peer reviewed publications presented the tissue repair potential of MSCs *in vivo*.

In the first study:

- Kinnaird et al.[33] utilised hMSC conditioned media (hMSCcm) and mMSC conditioned media (mMSCcm) to demonstrate paracrine mediated tissue repair and/or remodelling *in vitro* and *in vivo*. hMSCcm induced endothelial and smooth muscle cell proliferation and migration *in vitro* using multiple cytokine pathways, such as VEGF and PDGF-β respectively. The processes appeared to be regulated in a dose dependent manner.

[29] Devine SM, et al. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 101: 2999-3001.
[30] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.
[31] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.
[32] Ellison CA, et al. (2004) Effect of recombinant human keratinocyte growth factor (rHuKGF) on the immunopathogenesis of intestinal graft-vs.-host disease induced without a preconditioning regimen. *J Clin Immunol.* 24: 197-211.
[33] Kinnaird T, et al. (2004) Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 109: 1543-1549.

– In the mouse model of hindlimb ischemia, injection of mMSCcm to the adductor muscle resulted in improved flow recovery, less ischemic damage, reduced auto-amputation and improved limb function.

The above study shows the potential of hMSC and mMSC conditioned media to affect an *in vitro* and *in vivo* recovery process. As discussed below, some concerns remain regarding the biological relevance of this study:

- The number of hMSCs determines the concentration of cytokines produced; this allows for reasonable extrapolation of number of local engraftments required to elicit a cytokine effect.

In the second study:

- Sémont et al.[34] utilised the NOD/SCID mouse model to determine the effects of infused hMSCs ($5.0 \times 10^6$) on radiation induced intestinal injury. Using PCR based methods, 15 days after irradiation the authors demonstrated engraftment of hMSCs in the small intestine (0.17%), kidneys (0.1%) stomach, spleen (0.94%), liver (0.44%) and lung (0.82%). Higher percentage of MSCs appeared to be located in regions exposed to both, total body and local irradiation.

While Sémont et al.[34] shows the potential of hMSC to contribute towards regeneration of the GI tract post irradiation, questions remain regarding the outcomes of the study as an in vivo demonstration of tissue repair by MSCs:

- The authors observed increased intestinal villi height in non-irradiated animals treated with MSCs. Despite low engraftment rates, these observations demonstrated the effect of infused MSCs on the growth and turnover of the normal intestinal tissue.
- No data however was presented on histological analyses of other epithelial tissues from the non-irradiated group. If MSCs alone can promote intestinal tissue growth in the absence of damage, further studies on other tissues are warranted.While the benefit of a NOD/SCID model is understood when testing hMSCs, it would be beneficial to simultaneously compare and contrast outcomes on a normal mouse model or non-human primate model using allogeneic cell lines.
- The PCR based detection method, again, did not allow for histological localisation of grafted MSCs within the intestinal tissue structure; thus, it is difficult to ascertain if the observed effects are due to (a) direct involvement of MSCs in tissue regeneration with cellular contributions or (b) indirect support of endogenous cell proliferation through trophic support.

**Secondary pharmacodynamics and safety pharmacology**

Safely pharmacology involved investigation of cardiovascular and respiratory effects.

***Cardiovascular effects***

In a cardiovascular safety study, a range of doses per kg of swine MSCs (sMSCs) encompassing the clinical dose were infused into a swine model.

Two animals were euthanased prematurely due to complications arising from catheterisation. Six animals experienced catheter related adverse effects during the treatment period. The remaining subjects were terminated on scheduled necropsy dates.

[34] Sémont A, et al. (2006) Mesenchymal stem cells increase self-renewal of small intestinal epithelium and accelerate structural recovery after radiation injury. *Adv Exp Med Biol.* 585: 19-30.

Arrhythmias and tachycardia (>150 beats/min) events were noted pre infusion, mid infusion and post infusion; however, no correlation of these events was established to the test article.

Overall, the sMSCs were well tolerated in this safety study which was not designed to test biodistribution to cardiac muscle. Given that distribution of MSCs is stochastic and previous studies highlighted by the sponsor (Allers et al.[35]) have demonstrated the ability of infused MSCs to engraft to cardiac tissue; the absence of any specific heart muscle related findings may not necessarily be due to an absence of an effect on the tissue per se, rather absence of or insufficient amounts of sMSCs in heart tissue itself. As MSCs are expected to act locally, investigation of cardiovascular effects should be accompanied by biodistribution data and long term analysis of cardiac effects.

***Respiratory effects***

In a safety pharmacology study, male rats were exposed to high doses per kg (above the clinical dose) of rat MSCs (rMSCs) at different infusion rates to investigate respiratory changes.

Early deaths were reported in twenty-three percent of the animals following administration of the test article. Respiratory arrest was attributed to clumping of MSCs in the delivery system.

However, three additional in house acute toxicity studies did not record any infusion related deaths attributed to cell clumping. It is conceivable that differences in delivery method and testing (whole body plethysmography chamber, with a specialised swivel catheter (respiratory) versus femoral or jugular catheter (acute)) resulted in cell clumping.

The sponsor states loss of animals observed in the study could not be linked to concentration, dose or rate of cell infusion. While no correlation was established between each individual parameter, deaths were only reported in rMSC treatment groups.

Given that the cause of death was not clearly established at the NOAEL (No Observed Adverse Effect Level) recommended by the sponsor, their NOAEL on rat pulmonary function is not justifiable. Based on respiratory parameters only, a lower NOAEL based on this data is warranted. The study however did not report other clinical effects observed in another acute toxicity study at lower doses.

## Pharmacokinetics

### Absorption and plasma kinetics

The sponsor's assertion that biodistribution studies encompass absorption in cell therapies is acceptable. As such, separate absorption analyses are not required for the MSCs per se.

However, it is important to note that the MSCs secrete factors in response to cues from its local environment. When infused in sufficiently high and reasonably pure quantities, the molecules secreted by the cells are likely to have their own absorption characteristics and plasma kinetics, depending on number of parent stem cells located within any tissue. These characteristics of MSCs, which are exploited for their putative clinical benefit, were not interrogated in the studies presented in this submission.

[35] Allers C, et al. (2004) Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. *Transplantation* 78: 503-508.

Quantitative *in vitro* and *in vivo* analyses, involving absorption and plasma kinetics on key secretory factors relevant to clinical application of the test article is therefore recommended.

**Biodistribution**

Tissue distribution studies of the test article in healthy animals and irradiated models were presented in the form of three in-house studies and seven peer reviewed articles.

***Biodistribution: Healthy animals***

Two studies used colloidal cell tracking to tag and detect *in vivo* distribution of rMSCs. Tissue distribution was assessed from short term (hours) up to more than one week post infusion. In both studies, the presence of cells was confirmed in the lungs, kidneys, liver and spleen. In one study, fluorescently labeled infused cells were also detected in lung and liver; however, no cells were detected in spleen and kidneys. In the other study, no histological detection methods were used to confirm presence and/or location of labelled rMSCs within tissues.

While both studies indicated presence of infused rMSCs, the following concerns regarding the detection method are noted:

- A significant difference appears to exist between detection of cells using the two different techniques. No quantitative or semi quantitative analysis was performed using alternative histological or PCR based detection methods. It is, thus, difficult to assess reproducibility and accuracy without validation of the colloidal cell detection system
- No *in vitro* or *in vivo* histological detection methods were used to quantify the effects of the colloidal cell agent leakage from dead cells.

Liechty et al.[36] injected hMSCs to foetal sheep intraperitoneally (IP) at either gestation day (GD) 65 or 85. Tissues were harvested 2 weeks or 2, 5, or 13 months after injection. Using PCR, transplanted cells were detected in 28 or the 29 transplanted subjects (12/12 transplanted at 65 days and 16/17 transplanted at 85 days). Site specific chondrocyte, adipocyte, bone marrow, thymic, cardiomyocyte and skeletal myocyte differentiation of some grafted hMSCs was observed using immunohistochemical techniques. No quantitative data was available on the fraction of engrafted hMSCs.

While the study demonstrated incorporation of hMSCs into sheep foetuses, this model is not comparable to an adult system in which the test article is clinically applied:

- During gestation, embryos undergo rapid development; as such cells are exposed to many factors that promote development and differentiation. These signalling factors are largely absent in adult organisms. The extent to which the grafted hMSCs can survive, proliferate and/or differentiate in an adult organism is therefore likely to diminish due to reduced trophic support.

Gao et al.[37] used rMSCs to assess tissue distribution up to 48 h post infusion in 28 F-344 rats. A combination of intra artery (IA), IV and IP infusions were used with or without vasodilators.

Approximately 50% of labelled cells were distributed to the lungs following IA and IV infusion. Use of vasodilators increased label distribution to the liver by approximately 10% and simultaneously reduced signal intensity in lungs by approximately 15%. No

[36] Liechty KW, et al. (2000) Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nat Med.* 6: 1282-1286.
[37] Gao J, et al. (2001) The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. *Cells Tissues Organs* 169: 12-20.

difference was found in cellular distribution between IA and IV infusion methods, although in both instances, mobilisation to liver and kidneys was improved by inclusion of vasodilators. This study was specifically designed to address short term distribution of MSCs post infusion.

Allers et al.[38] infused four female nude mice with technetium 99m (99mTc) labelled hMSCs. Body scans performed 15 minutes to 24 h after infusion demonstrated presence of 99mTc signal in the lungs, heart, liver, kidneys and spleen. A further 20 animals infused with unlabelled hMSCs were assessed for presence of hMSC using PCR, which demonstrated presence of hMSCs in cardiac muscle, ear, tail cartilage, liver and teeth.

Based on the decrease in tissue distribution over a two week period, and subsequent increase in detection after 13 months, the authors suggest *in vivo* hMSC expansion (within bone marrow) and re distribution. At 13 months, using probes against human Alu sequence, hMSCs was detected in the bone marrow at an average frequency of in 1/580 cells (<0.2% total cell population). The phenotypic identity of the Alu+ cells was not addressed.

Though long term survival of hMSCs was reported, it must be noted that:

- Immune compromised mice were used in this study. While the model used is not comparable to the proposed clinical application of Prochymal it is an acceptable compromise. The biodistribution studies present a qualitative picture of MSC biodistribution in vivo. Collectively, the following concerns are noted regarding design and interpretation of data in these studies in healthy animal models.
- The cell labelling and detection methods utilised did not allow for detailed visualisation of cells within tissue; as such biodistribution is limited to the level of organs/tissues and not substructures.
- Large proportions of infused MSCs appear to be initially 'trapped' within vessels of the circulatory system. In order to retain the naive 'stem like' state, MSCs require tightly regulated niche environments. The absence of these regulatory environments within the vascular system could potentially impact on the survival, maintenance and differentiation of MSCs; ultimately affecting their intended functionality. This endpoint was not addressed by the studies.
- While labelled MSCs were detected in many organs/tissues, no quantification was provided on the number of cells embedded within tissue, excluding the vascular network.
- In control studies on healthy subjects, no data was presented on distribution of infused MSCs in the GI tissue; a major organ affected by GvHD. However results of MSC distribution or the absence of cells in GI tissues of healthy animals are not critical for safety and efficacy of MSCs in the GVHD indication, as all of the patients possibly receiving treatment are severely ill.

### *Biodistribution: irradiation models*

In an irradiation model, autologous canine MSCs (cMSCs) were infused in a canine model (25% controls and 75% TBI).

The cells were gene transduced for detection. Due to the transduction processes, cMSCs were placed in culture for extended periods, resulting in changes to *in vitro* morphology. No immunohistochemistry was performed to determine if morphology changes accompanied any change to the phenotype. The transduced gene was detected by

[38] Allers C, et al. (2004) Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. *Transplantation* 78: 503-508.

PCR/hybridisation in peripheral blood at a week in one subject and marrow at approximately 3 months in another subject.

While this was a pilot study, the following comments regarding the strength of the study are noted:

- No differentiation or homogeneity data on cMSCs prior to infusion were submitted.[39]
- *In vivo* bone/cartilage differentiation was only tested in immune compromised mouse models. No staining was observed when bone and cartilage formation was detected in explants. No contribution to bone formation from transplanted cMSCs was confirmed
- No *in vitro* differentiation studies were submitted; therefore, the differentiation potential and biological competence of the stem cell pool cannot be evaluated.

Mosca et al.[40] infused 10 myeloablated canine subjects with GFP transduced autologous cMSCs.

Necropsies were performed on Days 3, (n = 1), 14 (n = 1), 28 (n = 3), 60 (n = 3) and 182 (n = 2). Biodistribution was assayed using a quantitative PCR based method. In many tissue samples, cMSCs were detected at frequencies less than 0.1% of total cell population. In two subjects high GFP counts were observed in sample obtained from the femoral vein, peripheral liver and coronary artery. No signals were detected in the gut, brain, skin urinary or reproductive tissues. Levels of grafted MSCs exceeded 0.1% in at least 40% of the bone marrow samples tested.

The study indicates engraftment of infused cMSCs, but does not include:

- microscopy or histology analyses to validate and/or supplement the PCR-based findings
- location of grafted cells within target tissue or the *in vivo* fate of grafted cells
- the level of tissue damage caused by irradiation and distribution of cMSCs relative to the damaged tissue.

In the Devine et al. study,[41] 3 juvenile baboons, 2 of which conditioned with 1000cGy radiation and 1 control, were infused with either autologous MSCs (n=2) or allogeneic MSCs (n=1). Transplanted MSCs were transduced with GFP. The animals were sacrificed at 21, 19.5 and 9 months, respectively. Tissues were analysed for engrafted bMSCs using real time PCR against the transduced GFP sequence.

Both the autologous and allogeneic transplants demonstrated similar distribution patters. High levels of GFP were detected in the GI tracts of all three subjects. In addition, GFP was also detected in kidney, skin, lung, thymus and liver of all subjects. Overall, the non-irradiated recipient appeared to have fewer engrafted GFP positive cells as compared to irradiated recipients.

Data necessary to appropriately evaluate the strength of the stated findings were not provided and the PCR based findings were not based on a validated method. Furthermore:

- No histology data was provided on tissue damaged caused by irradiation and distribution of bMSCs relative to the damaged tissue

[39] Sponsor comment: 'These internal data were not submitted because this was never communicated to the sponsor as a question during the review process.'
[40] Mosca JD, et al. (2000) Mesenchymal stem cells as vehicles for gene delivery. *Clin Orthop Relat Res.* 379 Suppl: S71-S90.
[41] Devine SM, et al. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 101: 2999-3001.

- No data was presented regarding location of grafted cells within host tissue or the *in vivo* fate of grafted cells.

Chapel et al.[42] exposed 12 macaques to a combination of different BMMNCs and mcMSCs doses and different TBI conditioning regimes. A proportion of the test article was transduced with GFP for biodistribution detection.

Tissue samples were analysed for presence of test article using a genomic PCR against the GFP sequence. High levels of GFP signals were initially detected in bones, bone marrow, spleen, skin, skeletal muscle, tendon stomach and gut tissue within 23 days of irradiation. No GFP was detected in brain, liver, kidney, lung, cardiac muscle, thymus, rectum, and testicle within the same time frame.

While distribution of test article is detected in various tissues post infusion, concerns regarding the strength of the study include the PCR based findings were not based on a validated method and:

- No histology data was provided on distribution of mcMSCs relative to damaged tissue
- No data was presented regarding *in vivo* fate of grafted cells.

The above studies indicate a trend for MSCs to home towards tissues damaged by irradiation. However, the findings should be considered preliminary for the reasons discussed below:

- Most studies did not contain necessary controls to accurately compare biodistribution between irradiated and non-irradiated subjects. For example, in Chapel et al.,[43] the 3 non irradiated shams did not receive any MSCs; in Devine et al.,[44] the non-irradiated control received only autologous MSCs; and in an earlier study by Devine et al.,[45] the non-irradiated control did not receive any MSCs. In contrast, in the canine study in which appropriate controls were utilised, no appreciable biodistribution was observed. Collectively, these studies do not therefore provide strong evidence for increased engraftment of MSCs to tissues damaged by irradiation.
- Considerable variation was observed in biodistribution between different studies. Some variability is anticipated in terms of tissue penetration due to the nature of the test article. However, inability to detect MSCs in organs such as lungs and liver[46] within days of infusion suggests inconsistencies in distribution and/or detection.
- All studies utilised signal amplification based methods to detect presence of infused MSCs. As no alternative quantitative or semi quantitative detection methods such as immunofluorescence or immunohistochemistry were used, it was not possible to verify the accuracy and/or the reliability of the PCR based detection methods.
- In the absence of immunofluorescence or immunohistochemistry data it was also not possible to localise MSCs within host tissues and determine their post graft cell fates.

[42] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.
[43] Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.
[44] Devine SM, et al. (2003) Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 101: 2999-3001.
[45] Devine SM, et al. (2001) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol.* 29: 244-255.
[46] Devine SM, et al. (2001) Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Exp Hematol.* 29: 244-255; Chapel A, et al. (2003) Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *J Gene Med.* 5: 1028-1038.

### *Ectopic tissue formation*

An in house study infused cMSCs over a 10 fold cell dose range per kg into a canine model.

No ectopic bone or cartilage was detected using chest X rays up to approximately three months.

While this study implies lack of hard ectopic tissue formation, the following concerns regarding the study are noted:

- The cMSCs characterisation data analogous to Prochymal prior to infusion were not provided
- The study only examined for presence of hard tissue, such as bone or cartilage, and did not attempt to ascertain presence of ectopic soft tissue. (Note that Sémont et al.[47] observed increased intestinal villi height in non-irradiated animals treated with MSCs. Despite low engraftment rates, these observations demonstrated the effect of infused MSCs on the growth and turnover of the normal intestinal tissue).

The carcinogenicity/tumourigenicity section (below) further addresses ectopic tissue formation in the context of tumourigenesis.

### Pharmacokinetic drug interactions

Since hMSCs naturally exist within the human body, the sponsor claims that interference is unlikely with the pharmacokinetics (PK) of other drugs. It should however be noted that naturally occurring hMSCs are located within distinct cellular niches and are tightly regulated in their function by signalling processes within the niche. Prochymal in its clinical application form is expanded *in vitro*, and infused into the blood stream at concentrations much higher than normal circulation. As the hMSCs can collectively secrete high concentrations of different factors (some of which have immunomodulatory capabilities), it is difficult to conclude that interference with the PK of other drugs is unlikely, either systemically or locally.

## Toxicology

### Acute toxicity

The sponsor presented data from five single dose acute toxicity studies involving rat and canine models.

In a pilot study, autologous cMSCs over two orders of magnitude were infused into canine subjects (25% control and 75% TBI). No MSCs were detected in most tissues using the PCR/hybridisation based method targeting the transduced gene (detection sensitivity $1/10^5$ cells). MSCs were detected in one peripheral blood sample and one necropsy humeral marrow sample. Three Unanticipated Early Deaths (UEDs) were reported and attributed to complications associated with TBI. No test article related adverse pathological effects were noted in any concentration of MSCs infused. Based on the pilot study, the doses studied were identified as safe infusible doses.

Another study involved autologous infusion of cMSC at two doses, one order of magnitude apart, one day after TBI and bone marrow engraftment. Tissue distribution studies did not accompany the acute toxicity study. No test article related pathology was reported by sponsor. No ectopic bone or cartilage was detected using chest X ray detection. The serum

[47] Sémont A, et al. (2006) Mesenchymal stem cells increase self-renewal of small intestinal epithelium and accelerate structural recovery after radiation injury. *Adv Exp Med Biol.* 585: 19-30.

chemistry levels were reported as normal during and following infusion. The doses in this study were tolerated with no adverse effects.

A study in a rat model infused rMSCs over two orders of magnitude above the clinical dose at high dose volumes. Due to deaths immediately following infusion at the highest cell doses, approximately 10 fold and approximately 18 fold higher than the clinical dose, these groups were terminated. The cause of death was undetermined in most cases. Animals in all treatment groups including the cell free control showed red material around their retro orbital sinuses. A proportion of animals treated with concentrations higher than $8.0 \times 10^6$ cells/kg also showed impaired righting reflex and red discharge. No changes to bodyweight gain were observed. No obvious treatment related changes were observed in haematology or blood chemistry parameters. Cell viability in this study was low, ranging from 25-66%, and the study was therefore repeated.

Another study infused rMSC 5 fold above the clinical dose labelled with colloidal cell tracking agent or fluorescent label into two groups of rats. The rats were examined for adverse effects from short times through 10 days post dose. In 17% of the animals, up to 1 h post infusion, the following clinical symptoms were predominantly observed: skin cold to touch, reduced activity, and impaired righting reflex. The clinical signs resolved within 24 hours of treatment. No early UEDs were reported. The sponsor concluded observed clinical effects may have been due to the cell labelling methods used.

A rat toxicology study (pivotal) was conducted in two phases. In the dose range finding Phase A, up to approximately 40 fold greater than the clinical dose of rMSCs were infused into male rats. In Phase B, up to approximately 30 fold greater than the clinical dose of rMSCs were infused into two equal groups of male and female rats. No test article related deaths were reported. There were no clinical signs observed in the majority of the animals. Less than 10% of the animals at the highest cell doses showed clinical signs consistent with blood in the urine, which were deemed test article related. No test article related body weight changes were detected. No obvious treatment related changes were observed in haematology or blood chemistry parameters.

Based on these findings, the sponsor determined the NOAEL of $40.0 \times 10^6$ rMSC/kg and MTD of $65.0 \times 10^6$ rMSC/kg. However, as discussed below, concerns remain regarding the overall strength of acute toxicity data provided:

- In this series of single dose toxicity studies, the sponsor observed different minimal dose levels at which similar clinical effects were observed.
- Difficulty was experienced in comparing outcomes of the single dose toxicity studies as detailed cell homogeneity, culture conditions and differentiation potential data were not provided for all studies.
- The sponsor indicated that the effects observed may have been a result of either differences in production methods or cell labelling agents. As no internal controls were available, it is difficult to accurately reconcile the discrepancy. This suggests variability between experiments or potentially, different batches of rMSCs.
- Considering the large variability, and based on the data provided, a suitable NOAEL would likely be lower than the proposed NOAEL, and significantly closer to the proposed clinical dose.

**Repeat dose toxicity**

Data from two repeat dose studies using rats were presented.

A pilot study utilised doses up to 25 fold greater than the proposed clinical dose infused into a rat model. The test article or the vehicle control was administered to all groups by IV infusion over a two week period for a total of five sequential doses of 5 mL/kg/dose.

Six UEDs occurred at the highest dose level. While no definite cause of death was established, all UEDs had thrombi in various organs based on microscopic analysis. No microscopic analyses were however performed for scheduled necropsies for comparative purposes. Test article related clinical effects were noted at the highest dose. These observations included, breathing difficulties, decreased or impaired function, cold skin, and protruding eyes. Discharge and discoloured urine was detected in all groups, including the control, therefore the vehicle was implicated. No test article related effect on haematology, urinalysis, or clinical analyte parameters was observed.

In a pivotal study, the test article or control vehicle was administered over a two month time period for a total of 13 doses per animal at 5 mL/kg/dose.

There were no clinical findings or deaths up to a cumulative dose of 80 x $10^6$ rMSCs/kg.

In less than 3% of the animals, UEDs were reported (half immediately following infusion in main study, one quarter in an immunotoxicity satellite study, and one quarter at one month post infusion). The cause of death for most animals was undetermined; the death of one male the day after administration was attributed to the test article. No control (vehicle only) animals died during the repeat dose study. In less than 5% of the animals, test article related clinical effects were observed at the two highest doses. The clinical effects included red discharge and decreased or, impaired function or activity, and pale skin.

Test article related emboli (minimal to mild) were detected in lungs at all dose levels. Minimal to mild thrombi were observed, which were also considered rMSC related. The presence of emboli and thrombi were considered reversible as the size and incidence were lower at necropsy.

No test article related haematology effects were observed. Statistically significant changes in blood lymphocytes subsets were observed in treated animals. However, as the values were considered within normal biological variation, the sponsor concluded that there no test article related changes in peripheral blood lymphocyte subsets.

To this end, it must be noted that Prochymal has unique characteristics, and in this context, the scope of toxicity studies is limited as discussed below:

- The repeat dose toxicity studies did not utilise hMSCs; instead a rat equivalent of the test article was used. Rat MSCs were similar, but not identical to hMSCs in terms of manufacture and characteristics. Prochymal hMSCs are positive for the phenotype markers CD105 and CD166, but commercial antibodies against rat CD105 and CD106 were not available, hence CD73 and CD90, which have been recommended as minimal MSC markers,[48] were used. Alternatively, or additionally, the toxicity studies could have been conducted on comparable species, such as non-human primates. It is unclear whether the lung emboli observed in rats are restricted to small animal species. The sponsor stated that they were unaware of any reports of lung emboli in large species or humans.
- In the absence of gene expression analysis, it is difficult to establish if the genetic homogeneity of the test article was consistent between each dose.Changes to organ weights were observed during the repeat dose study, however none of these organ weight changes were associated with microscopic findings. They were not considered adverse. Data on biodistribution of rMSCs to target organs was not investigated in this study. Given the proposed local action, inclusion of biodistribution and *in vivo* phenotypic data in the toxicity studies are recommended.

[48] Dominici M, et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.

**Carcinogenicity/tumourigenicity**

A standard six week tumourigenicity study was performed with hMSC in the nude mouse model. In addition, data from three large animal studies were also presented as preclinical evidence for lack of tumourigenicity of MSCs.

A developmental preparation of hMSCs was SC injected at approximately 16 fold the proposed clinical dose nude mice of 8-20 weeks of age. No tumours or other lesions related to the test article were detected after 6 weeks.

Another study infused cMSCs at two doses or placebo over an order of magnitude in a canine total body irradiation model. No ectopic bone or cartilage was detected using chest X-rays in animals followed for three months.

A study of female baboons infused bMSCs IV (allogeneic, donor X) followed by intramuscularly (IM) administered bMSCs (allogeneic, donor X and donor Y). The cell doses were on the order of the proposed clinical dose. A range of tissues was sampled at approximately 6 months for microscopy. Benign tumours were identified in three bMSC treated subjects; of these, two were detected only in a microscopic examination. As the baboons were 11-14 years of age, the tumours were deemed to be age related. It should be noted that the Final Pathology Report states that:

**'The group size was too small to permit assessment of oncogenic effects of test article' (Page 6).**

No data was provided on tissue distribution of bMSCs. No tumours were reported in control subjects.

A swine study infused sMSCs over an order of magnitude in a swine model of approximately 6 months duration. No evidence of carcinogenicity was reported in this study.

The sponsor performed a single 6 week tumourigenicity study using hMSCs from a developmental product in the nude mouse model. Further evidence for absence of test product carcinogenicity was provided based on short term non carcinogenic large animal studies. However, no long term and comprehensive carcinogenicity studies were performed by the sponsor citing the relevant ICH guideline:[49]

**'Standard carcinogenicity bioassays are generally inappropriate for biotechnology-derived pharmaceuticals. However, product-specific assessment of carcinogenic potential may still be needed depending upon duration of clinical dosing, patient population and/or biological activity of the product (e.g., growth factors, immunosuppressive agents, etc.) When there is a concern about carcinogenic potential a variety of approaches may be considered to evaluate risk.'**

Given the preliminary nature of the findings and biological activity of the test article *in vivo*[50], the need for additional carcinogenicity studies is discussed below:

- One study used a MSC concentration comparable to the maximum cumulative clinical dose. However, the study spanned only 3 months. Further carcinogenicity studies may be warranted at appropriate clinical doses over the long term for other indications.

[49] European Medicines Agency, 'ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals: Step 5 (EMA/CHMP/ICH/731268/1998)', June 2011.
[50] Sponsor comment: 'We stated that there were not standard animal models available that are applicable for carcinogenicity testing. The applicability of animal studies in the case of a cell therapy is limited. This application for the treatment of a severe, ultra orphan disease like GvHD, necessarily relies heavily on the clinical safety results'.

**Reproductive toxicity**

Given the poor survival prognosis for steroid refractory aGvHD and the absence of detectable grafted cells in gonads, the sponsor did not consider standard reproductive and development toxicity studies necessary, which is acceptable.

Advice is sought from the Advisory Committee on Prescription Medicines (ACPM) as to whether an Australian pregnancy category (B3) is warranted for Prochymal, the first adult stem cell product proposed for registration.

**Immunotoxicity**

The potential for immunotoxicity was investigated in the pivotal repeat dose toxicity study in rats, and an immunotoxicity study (single dose) in female baboons.

The effect of the test article on immune response was measured by the ability of injected rats to generate antibody in response to an antigen. Peripheral blood lymphocyte subsets examined 13 weeks after the first dose showed some changes in NK cells, mean lymphocyte levels, T cells, CD4+ and mean B cell counts between males and females when compared against controls. The sponsor deemed the changes in lymphocyte counts to be within normal biological variation, thus no test article related effect was attributed to changes seen in peripheral blood lymphocyte subsets.

Splenocyte proliferation assays were performed to determine the effects of test article on immune cell functionality. Based on results of the splenocyte assay, it was concluded splenocytes of the treated groups were 'primed' or 'preconditioned' to be more proliferative as a result of treatment. As the endpoint readings for both male and female samples were comparable with vehicle controls, the sponsor concluded no test article related effect on splenocyte proliferation.

The ability of the infused rats to generate alloantibodies was assessed using fluorescence conjugated secondary antibodies. Alloantibodies were detected in all dose levels and many dilutions; however, no corresponding histopathological findings were observed. At 1:1000 dilution of the $0.2 \times 10^7$ cells/kg dose, alloantibodies were also not detected. These findings were interpreted as a low alloantibody titre by the sponsor, and therefore of no biological significance.

In a female baboon study, allogeneic bMSCs were infused and injected. Lymphocyte analysis revealed minimal, but statistically significant changes to CD3+/CD4+ in four of the groups examined. While the percentage of CD3+/CD4+ and CD3+/CD8+ cells were different, their ratios were maintained and the total number of lymphocytes was unchanged. No treatment related changes to antinuclear antibodies and IgG panel (IgG and IgM) were detected.

Overall, no immunotoxicity effects were attributed to the test article. While impact of test article administration on immunotoxicity appeared minimal, the following concerns are noted:

- Only characteristics of rat MSCs were provided. No data was provided on passage numbers, homogeneity or viability of injected baboon cells
- The baboon study utilised labelled cells. No data was provided on the biodistribution of infused cells. Therefore, in the absence of engraftment/uptake data, it is difficult to contextualise the observations in the immunotoxicity studyIn the rat study, it should be noted however that while the final proliferation measure between vehicle control and treated cells are comparable, the magnitude of change in proliferation between the two groups are not comparable. In both males and females, the fold change in the mean % reduced figure after stimulation is less in treated splenocytes when compared to control, potentially implying a reduced response in treated splenocytes

- Both studies did not provide data on level of activity of rMSCs or bMSCs using *in vitro* techniques, such as MLRs or cytokine secretion profiles. The data provided focuses on physical concentrations of MSCs that can be utilised, but not the activity profiles of the MSCs at the time of infusion.

### Local tolerance

Mild irritation was noted in injection site of both male and fe*males in* the repeat dose toxicity studies.

### Excipients

#### *DMSO*

Prochymal is formulated with DMSO, the most common cryoprotectant. DMSO is commonly used for IV delivery of hematopoietic stem cells for more than 40 years of bone marrow transplantation history. The DMSO concentration in Prochymal is reduced when diluted with Plasma-Lyte medium prior to infusion. The cells are not washed to remove DMSO prior to administration.[51] The Prochymal dose is 2 x $10^6$ hMSC/kg, or one bag of Prochymal for a 50 kg patient (1.65 g DMSO per 15 mL bag), hence the single dose of DMSO is 33 mg/kg body weight. Patients will be treated with Prochymal twice weekly for 4 weeks.

DMSO has been reported to have cardiovascular effects in animals, and humans.[52] The sponsor submitted a GLP compliant safety pharmacology study which investigated the potential for cardiovascular effects in swine. The original purpose of this study was to assess acute and long term safety of sMSCs, for a cardiac indication. Swine in the control group were IV infused with the vehicle, at an infusion rate of 2-3 mL/min, and observed through approximately 6 weeks. The DMSO dose corresponded to approximately 78 mg/kg body weight. No treatment related cardiovascular or other toxicities were observed.

Other reported effects of DMSO in humans are nausea, vomiting, elevated liver enzymes, and rare cases of neurological toxicity.[53] The clinical evaluator may wish to comment on the reported human adverse effects of DMSO.

#### *Human serum albumin (HSA)*

Prochymal contains HSA, which serves as a cell stabiliser.

#### *Plasma-Lyte A*

Prochymal contains 70% Plasma-Lyte A physiological medium. The Australian equivalent, Plasma-Lyte 148, is entered on the ARTG.

### Cell and protein impurities

The drug product specifications include a limit for CD45 positive cells, which are of haematopoietic origin, and may contain unmatched donor T cells. Published clinical data[54] have estimated that T cells in numbers <1 x $10^5$/kg body weight in bone marrow or in

[51] Windrum P, et al. (2005) Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres. *Bone Marrow Transplant.* 36: 601-603.
[52] Horacek JM, et al. (2009) Cardiovascular changes associated with infusion of hematopoietic cell grafts in oncohematological patients -- impact of cryopreservation with dimethylsulfoxide. *Exp Oncol.* 31: 121-122.
[53] Júnior AM, et al. (2008) Neurotoxicity associated with dimethylsulfoxide-preserved hematopoietic progenitor cell infusion. *Bone Marrow Transplant.* 41: 95-96.
[54] Kernan NA, et al. (1986) Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. *Blood* 68: 770-773.

peripheral blood HSCT are unlikely to cause GvHD. The Prochymal cell dose is 2 x $10^6$/kg body weight, hence the maximum possible number of T cells per dose is more than 6x lower than the threshold for GvHD. The sponsor considered that T cells, which are non-adherent, were likely to be reduced in the manufacture of Prochymal. As indicated by FACS data showing absence of CD454 positive cells.

### Use in children

Prochymal is proposed for use in patients ≥6 months of age. No nonclinical studies in young animals were submitted.

## Nonclinical summary and conclusions

### Summary

- The sponsor has proposed to register Prochymal, a formulation of *ex vivo* adult hMSCs, for rescue of patients ≥6 months of age with aGvHD refractory to treatment with systemic corticosteroid therapy, or other immunosuppressive agents. The hMSCs are derived from the bone marrow of unrelated and HLA unmatched healthy adult donors.
- The 15 mL Prochymal formulation contains approximately 100 x $10^6$ viable cells in Plasma-Lyte A medium containing 5% HSA and 10% DMSO. The thawed product is diluted in 25 mL of Plasma-Lyte A (total volume 40 mL) prior to use.
- The proposed dosing regimen involves IV administration of hMSCs at 2.0 x $10^6$ cells/kg body weight, at 4-6 mL/min, for patients weighing 35 kg and over. For patients weighing less than 35 kg Prochymal should be infused over the course of 60 min. Patients are to be treated with Prochymal twice weekly (at least 3 days apart) for 4 weeks. If the response at 4 weeks is unsatisfactory, treatment may be continued with 2 x $10^6$ hMSC/kg once a week for 4 weeks. If GvHD recurs after 8 weeks treatment, a second treatment cycle may be initiated.
- No efficacy studies were provided in animal models of aGvHD. Primary pharmacology data consisted of published *in vitro* immunomodulatory, and in vivo tissue homing, protection and repair studies. Though *in vitro* immunomodulation by MSCs was demonstrated in MLR assays, the studies had minimal physiological context. Immunomodulation *in vivo* was demonstrated using a baboon skin graft model.
- *In vivo* tissue homing was assessed in irradiated macaque and baboon models. A slight trend towards increased MSC accumulation was observed within tissue damaged by irradiation in one published study. However, limitations in experimental design and animal numbers hindered quantitative analysis of MSCs homing to irradiated tissue. The study data did not allow for *in situ* identification of the cellular fate of the transplanted cells. *Safety* pharmacology studies investigated respiratory effects in rats, and cardiovascular effects in pigs. In the rat study, deaths were reported only in the treatment group, which were putatively attributed to cell clumping in the delivery system, but not confirmed. No other clinical symptoms were noted. No test article related clinical symptoms were noted in the porcine cardiovascular study during or immediately post infusion. While ECGs (electrocardiograms) were performed up to 24 weeks post infusion, the presence of test MSCs in cardiac tissue was not studied. There are sporadic, published clinical reports of systemic effects (nausea, vomiting, cardiovascular, respiratory) during DMSO infusion.
- Biodistribution data from irradiated and non-irradiated animal models was presented as a combination of publications and in house studies. In non-irradiated mouse, rat and foetal sheep studies, the infused MSCs were detected in different tissues.

Distribution patterns were stochastic and level of incorporation into tissue detected was very low (often less than 1%).

- In irradiated canine (beagle), macaque and baboon studies, infused MSCs were also detected in different tissues. MSC incorporation in tissues was at very low levels, and biodistribution variable between different studies.
- Tissue incorporation of infused cells was detected in all studies; however, the test methods did not facilitate characterisation of grafted cells *in situ*. The presented data did not provide strong evidence for increased engraftment of MSCs to tissues damaged by irradiation, and thus by extension, tissues that are damaged by GvHD.
- Acute dose toxicity studies performed using rat and canine MSCs resulted in NOELs with up to 4 fold variation in dose within species. The most common clinical symptoms were breathing difficulties, impaired righting reflexes and red material. Based on acute toxicity data, the nonclinical evaluator proposed a revised NOAEL 1.5 fold greater than the maximum clinical dose.
- Repeat dose toxicity was characterised in two (one pivotal) rat studies via the clinical route. No notable clinical effects were observed at the proposed Prochymal dose range. Test article related clinical effects were noted in less than 5% of animals such as breathing difficulties and decreased function, and red material at high doses of rMSCs. Repeat dose toxicity was only performed in one rodent species, no non rodent repeat dose studies were performed as stipulated by ICH guidelines.[55] No toxicity studies were conducted in young animals.
- No test article related tumourigenic potential was noted with a developmental hMSC product in a 6 week tumourigenicity study (non GLP) in nude mice, or three large animal studies. No long term carcinogenicity studies were performed by the sponsor, citing ICH Guideline S6:[56]
- **'Standard carcinogenicity bioassays are generally inappropriate for biotechnology derived pharmaceuticals'**
- In consideration of published literature indicating involvement of MSCs in tumour growth and/or propagation, the nonclinical evaluator believes that further long term carcinogenicity studies are warranted.
- Given the poor survival prognosis for steroid refractory GvHD and the absence of detectable grafted cells in gonads, the sponsor did not consider standard reproductive and development toxicity studies necessary, which is acceptable. Advice is sought from the ACPM as to whether an Australian pregnancy category (B3) is warranted for Prochymal.
- Mild local irritation was noted at injection sites in the repeat dose toxicity studies. No immunotoxicity effects were attributed to the test article.
- Overall, in many of the presented studies, information regarding specific culture conditions, such as, passage numbers, homogeneity of culture, *in vitro* differentiation potential and cytokine activity profile of cells were not provided. Variability of these parameters may be attributed to the lack of consistency observed between different studies. Presentation of these parameters is essential for accurate understanding and interpretation of data provided.

[55] European Medicines Agency, 'Committee for Proprietary Medicinal Products (CPMP): Note for Guidance on Repeated Dose Toxicity (CPMP/SWP/1042/99)', 27 July 2000.
[56] European Medicines Agency, 'ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals: Step 5 (EMA/CHMP/ICH/731268/1998)', June 2011

**Conclusions and recommendation**

- It is acknowledged that based on existing evidence, the immunomodulatory properties of MSCs could potentially be of benefit to patients suffering from GvHD. The evidence set forth in the submitted dossier however is limited in depth and breadth in terms of mechanism of action, efficacy and safety studies. Major limitations were imposed by the need to use homologous animal models, due to the likelihood of immune reactions against the human cells, and limited information on the relevance of the animal models to humans.
- The primary pharmacology studies demonstrated potential for immunomodulation *in vitro*, but with limited physiological context. *In vivo* immunomodulation studies did not utilise a GvHD model which is understood to be difficult to generate in rats. Evidence for *in vivo* tissue homing was ambiguous due to limitations in experimental design and test subject numbers. Data presented on tissue protection and repair did not on the whole use adequate analysis when MSCs were tested. Taken together, the primary pharmacology data provide limited evidence of Prochymal efficacy for the proposed indication. As such, it is not possible to advice on dose or dose regimen, nor on the potency assays, on the basis of the nonclinical data.
- Secondary pharmacodynamics (PD) and safety pharmacology studies investigating cardiovascular and respiratory effects did not identify any direct clinically relevant safety hazards. As discussed in the Assessment, respiratory effects related to delivery of the test article were however noted in rats. There are however published clinical reports of sporadic systemic effects (nausea, vomiting, cardiovascular, respiratory) during DMSO infusion.
- Acute and repeat dose toxicity studies demonstrated adverse effects on the lungs in relation to the infusion process (physical size of rat stem cells). Considerable variability in dose levels and clinical symptoms were observed between studies, which may be due to variability in processing of the test article. No toxicity studies were conducted in young animals.
- A developmental hMSC product did not show any tumourigenic potential in a short term study in nude mice. No standard long term tumourigenicity studies were performed per se. The sponsor claimed, citing ICH Guideline S6:[57]
- **'Standard carcinogenicity bioassays are generally inappropriate for biotechnology derived pharmaceuticals'**
- In consideration of published literature indicating involvement of MSCs in tumour growth and/or propagation, the nonclinical evaluator believes that further carcinogenicity/tumourigenicity studies are warranted in the future.
- No reproductive studies were performed with Prochymal, due to the poor survival prognosis of patients. Advice is sought from the ACPM as to whether an Australian pregnancy category (B3) is warranted for Prochymal, the first adult stem cell product proposed for registration.
- Overall, the majority of the studies did not utilise Prochymal in its clinical formulation.
- In view of these and numerous other outstanding concerns described in this evaluation report, approval for Prochymal is not supported on nonclinical grounds based on the data submitted.

[57] European Medicines Agency, 'ICH guideline S6 (R1) – preclinical safety evaluation of biotechnology-derived pharmaceuticals: Step 5 (EMA/CHMP/ICH/731268/1998)', June 2011

# IV. Clinical findings

## Introduction

The clinical dossier included two clinical efficacy and safety studies supporting the proposed indication, but no PK or PD studies in humans.

The submission included the following clinical information:

- One Phase 3 study in 260 adult and paediatric patients (Study 280)
- One statistical analysis of the paediatric subpopulation (n=28) enrolled in Study 280
- One expanded access study in 59 paediatric patients (Study 275)
- Single use reports of Prochymal in 12 patients
- Two cardiac safety synopses (reports 401 and 402)
- Case report forms (CRFs) and individual patient listings for Studies 280 and 275, and single patient use CRFs and
- Literature references.

See CER Attachment 2 for further details of the submitted studies and patient numbers.

### Paediatric data

The submission includes data from studies undertaken in both paediatric and adult patients with refractory aGvHD. Osiris states that paediatric subjects have been a part of the Prochymal clinical development plan since its inception. Consequently, a separate clinical development plan for paediatric patients has not been required. Study 275 included paediatric patients (n = 59) only and Study 280 (paediatric and adult population) included a subgroup analysis of paediatric patients (n = 28). Based on the contents of the Clinical Overview and the Summaries of Clinical Efficacy and Safety, it appears that the submission has been prepared to support a paediatric indication only, rather than a paediatric and adult indication.

### Good clinical practice

The studies in the submission sponsored by Osiris are stated to have been 'completed according to the guidelines of Good Clinical Practice including the archiving of essential documents. Compliance with this standard provides public assurance that the rights, safety, and well-being of trial patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki'.

## Pharmacokinetics

There were no Prochymal PK data in humans in the submission. PK studies for Prochymal are limited to tissue distribution studies in animals. Osiris states that suitable techniques are not currently available to measure the distribution of hMSCs in patients with GvHD. Preclinical studies are stated to have demonstrated that hMSCs clear from the blood within hours of administration. The cells initially distribute to the lungs within minutes of infusion, and at 24 h post infusion a majority of the cells are found in the lungs with lesser amounts in the liver.

### Pharmacodynamics

There were no Prochymal PD data in humans in the submission. The PD of Prochymal have been studied only in animals. Osiris states that there are no techniques currently available to measure the PD of Procyhmal in humans. Consequently, the effects of Prochymal in humans are assessed through clinical outcomes.

### Dose selection

Initial studies with MSCs reported promising results in paediatric and adult patients with steroid refractory Grade III or IV aGvHD.[58] In 16 patients (14 acute and 2 chronic GvHD), the median dose was 1 x $10^6$ cells/kg (range 0.4 to 9 x $10^6$ cells/kg), with the dose being administered from 1 to 3 times (9 patients received 1 dose, 6 patients received 2 doses, and 1 patient received 3 doses).

Based on the encouraging results from the initial studies with MSCs in aGvHD, Osiris undertook an open label, Phase 2 study in adults aged 18 to 70 years (inclusive) with adult GvHD (Grade II-IV) to determine whether the addition of Prochymal at a dose of 2 or 8 x $10^6$ MSCs/kg to corticosteroid therapy would improve patient outcomes.[59] Two infusions of Prochymal were administered, with the first being given 24 to 48 h after the diagnosis of aGvHD and the second being given 3 days after the first infusion. The primary efficacy endpoint was the proportion of patients who achieved complete response (CR) of aGvHD by study Day 28. The study found that 66.7% (10/15) of patients treated with the high dose had a CR compared with 87.5% (14/16) of patients treated with the low dose. There were a number of secondary efficacy endpoints including, partial response (PR), time to best response, addition of escalated immunosuppressive therapy, and survival through to study Day 90. Overall, the study showed that the low dose of Prochymal (2 x $10^6$ MSCs/kg) appeared to be as effective as the higher dose (8 x $10^6$ MSCs/kg) in inducing a response. The response criteria in this study were consistent with those in Study 280.

While Kebriaei et al.[60] was not designed to assess the optimal dose and schedule of Prochymal administration, 5/24 (20.8%) patients who achieved an initial CR had an aGvHD flare requiring second line therapy during the first 28 days following initiation of treatment. These GvHD flares suggested to the authors that 2 doses of Prochymal may be insufficient to maintain a CR. Furthermore, the authors noted that Le Blanc et al.[61] had found that multiple infusions were needed to achieve a sustained response in more that half of the patients treated with MSCs for steroid refractory GvHD. In view of the reported clinical experience in single use protocols suggesting that more than 2 infusions would be required for patients with more severe forms of aGvHD, Osiris specified that an initial treatment regimen of 8 infusions of 2 x $10^6$ cells/kg over a 4 week period were to be used in Studies 280 and 275.

> **Comment:** The submission included no formal dose finding studies. In Kebriaei et al.,[62] an inverse relationship was observed between MSC dose and CR at 28 days with the lower dose (2 x $10^6$ MSCs/kg) showing a greater response than higher

[58] Le Blanc K, et al. (2004) Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 363: 1439-1441; Le Blanc K, Ringdén O. (2006) Mesenchymal stem cells: properties and role in clinical bone marrow transplantation. *Curr Opin Immunol.* 18: 586-591;
[59] Kebriaei P, et al. (2009) Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 15: 804-811.
[60] Kebriaei P, et al. (2009) Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 15: 804-811.
[61] Le Blanc K, et al. (2008) Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 371: 1579-1586.
[62] Kebriaei P, et al. (2009) Adult human mesenchymal stem cells added to corticosteroid therapy for the treatment of acute graft-versus-host disease. *Biol Blood Marrow Transplant.* 15: 804-811.

dose (8 x $10^6$ MSCs/kg). However, the study was not designed to assess the optimal Prochymal dose and administration regimen.

## Efficacy

### Overall comments

It is considered that the submitted data do not support the efficacy of Prochymal for the rescue of patients ≥ 6 months of age with a GvHD refractory to treatment with systemic corticosteroid therapy or other immunosuppressive agents. The pivotal study is considered to be Study 280, and the analyses of the primary and secondary efficacy endpoints in this study showed no statistically or clinically significant differences between Prochymal and placebo. The study was multinational, multicentre, randomised, placebo controlled and double blind in design and included a total of 260 patients analysed for efficacy across the age range covered by the proposed indication. The mean ± SD age of patients in the study was 42.6 ± 17.2 years, and the majority of patients were aged ≥ 18 years (89.2%; n = 232).

The supportive study is considered to be Study 275, a multinational, multicentre, Prochymal single arm study in 59 paediatric patients aged < 18 years (mean ± SD age 8.4 ± 6.0 years, range 0.2 to 17.5 years). However, the data from this study is considered to be difficult to meaningfully interpret due to the absence of a placebo control arm. Therefore, Study 275 is considered to provide inadequate evidence supporting the efficacy of Prochymal for the proposed indication in the proposed population. The efficacy data from the Clinical Study Report (CSR) (BB-IND No. 7939-Prochymal) relating to 12 paediatric patients treated under single patient, emergency use protocols are of interest only, and are not considered to be relevant to the regulatory decision to approve or reject the application to register Prochymal for the proposed indication.

In documents submitted by the sponsor, Study 275 was nominated as the pivotal study, although it included only paediatric patients aged less than 18 years. From the content of the Clinical Overview, and the Clinical Efficacy and Safety Summaries, it appears that the submission was primarily prepared to support Prochymal for rescue treatment of steroid refractory aGvHD in a paediatric population (that is, patients aged < 18 years) rather than in both paediatric and adult populations (as proposed for Australia). For the purposes of the Australian submission, Study 275 should be considered to be supportive rather than pivotal as it does not include the complete age range of patients proposed for treatment with Prochymal covered by the proposed Australian indication.

Study 275 was a single arm study, and the absence of a placebo control arm makes interpretation of the data difficult. Osiris stated that Study 275 did not include a placebo control for 'ethical reasons', and instead a 'historical control was employed for the comparison of survival'. The historical control was used to evaluate the 180 day survival data, and there was no historical control to aid evaluation of the pre specified primary and secondary efficacy endpoints. In Study 280, the choice of the placebo control group was justified by the sponsor because both active and placebo treatment groups also received the institutionally defined standard of care (for example, a second line therapy in addition to continued corticosteroid treatment). However, Study 275 also allowed standard supportive therapy for GvHD to be administered at the Investigator's discretion and in accordance with site specific policies. As discussed above, it appears to be inconsistent to state that a placebo control was not considered to be an option for 'ethical reasons' in Study 275, but was permitted in Study 280. There is information in the submission suggesting that Study 275 was initiated at the request of physicians who specifically wanted to treat their patients with Prochymal rather than enrol them in Study 280 and risk randomisation to placebo.

The difficult of interpreting the results of the Study 275 without a placebo control can be illustrated by examination of the primary efficacy endpoint in this study of overall response (OR) at Day 28. The OR rate at Day 28 in Study 275 was 62.7% (37/59) and the non-responder rate was 37.3% (22/59). In Study 275 it is stated that 'due to the refractory nature of this population [that is, patients with aGvHD refractory to corticosteroids] achieving any response in these patients is meaningful and unexpected' (CSR). However, the overall response rate at Day 28 in Study 280 was 57.7% (94/163) in the Prochymal group compared with 50.6% (41/81) in the placebo group, p = 0.224. Consequently, from the Study 280 results it can be concluded that a meaningful overall response at Day 28 was obtained not only for patients treated with Prochymal, but also for those who received placebo in addition to standard of care (high dose immunosuppressives). Therefore, it is considered that the results for overall response at Day 28 from Study 280 do not support the statement in Study 275 that any response to Prochymal in patients with aGvHD refractory to corticosteroids is 'meaningful and clinically unexpected'. Furthermore, in Study 275 the overall response (OR = CR + PR) at Day 28 was based on patient assessments between study Days 20 and 38 rather than 28 ± 2 days as specified in the protocol. Consequently, the reported OR at 28 days in Study 275 might be an overestimation of the protocol specified response.

The difficulty of interpreting the results of Study 275 without a placebo control can be further illustrated by examination of the 180 day survival data. In Study 275, patients treated with Prochymal had a statistically significantly higher probability of survival through to 180 days (post onset of aGvHD) compared with patients in the historical control group (p = 0.003). The effect size was most pronounced in the aGvHD Grade D population (historical control: 31.0% [95% CI {Confidence Interval}: 26.0-36.0] versus Prochymal 56.2% [CI: 40.5-71.8]). In Study 280, survival > 180 days (post study start) in the overall aGvHD (Grade B-D) population was higher in the placebo group than in the Prochymal group (42.0% [34/81] versus 34.4% [56/163], respectively; p = 0.274). Furthermore, in Study 280 the corresponding results for the aGvHD (Grade C/D) population was 30.2% (28/126) in the Prochymal group and 40.0% (24/60) in the placebo group.

Despite the Study 275 results for 180 day (post onset of aGvHD) survival data showing significant superiority of Prochymal compared with a historical control, the Study 280 results did not show the same effect at a later point in the disease course. In considering the survival data in Study 280 Osiris concluded that, 'once past the treatment phase and especially beyond Day 100, there are many competing risks for this severely ill patient population with a life threatening disease, which cannot be controlled within the framework of a clinical trial. These confounding factors make it difficult to evaluate survival at later times' (CSR).

**Study 280**

In Study 280, there was no statistically significant difference between Prochymal and placebo for the pre specified primary efficacy endpoint of complete response sustained for ≥ 28 days in the ITT (Intention To Treat) population: 34.7% (60/173) and 29.9% (36/87), respectively, p=0.423. The study was powered on a complete response rate of 29% in the placebo group and 49% in the Prochymal groups. Consequently, it can be inferred that the absolute difference of 4.8% between the Prochymal and placebo groups observed for complete response sustained for ≥ 28 days is clinically non significant. In addition, there were no statistically or clinically significant differences between Prochymal and placebo for the pre specified secondary efficacy endpoints in the modified ITT (mITT) population of survival post infusion for greater than 100 days (52.1% versus 50.6%, respectively, p = 0.780), and for greater than 180 days (34.4% versus 42.0%, respectively, p = 0.274). There were also no clinically meaningful differences between the two treatment groups in

average corticosteroid use from post infusion through to Day 56 computed at weekly intervals (descriptive statistics only presented). Overall, the analyses of the pre specified primary efficacy endpoint (complete response rate sustained for a duration of ≥ 28 days) and secondary efficacy endpoints (survival status at 100 and 180 days) are considered to be robust, and unequivocally demonstrate that the observed differences between Prochymal and placebo are not statistically or clinically significant.

Despite the robust primary and secondary efficacy endpoint analysis demonstrating no significant difference between Prochymal and placebo Osiris states that 'given the inherent challenges associated with conducting a large, controlled trial in steroid refractory GvHD, these analyses (the additional and/or exploratory efficacy analyses discussed below) provide placebo controlled evidence that Prochymal results in a clinical benefit to patients with visceral organ steroid refractory GvHD' (CSR). In coming to this conclusion, the sponsor appears to give more weight to the evidence from additional and/or exploratory efficacy analyses than to the pre specified primary and secondary efficacy analyses. This approach is considered to be unacceptable for the regulatory purpose of determining whether Prochymal should be approved for the proposed usage in the proposed patient population. From a regulatory perspective, it is considered to be methodologically unsound to emphasise the importance of the efficacy results from additional and/exploratory endpoint analyses when the pre specified primary and secondary efficacy analyses unequivocally fail to demonstrate the significance of the investigational agent compared with placebo.

In discussing the efficacy conclusions, Osiris contends that the study 'had a rigorous primary endpoint – a complete response, meaning complete resolution of all clinical signs of GvHD – that had to be maintained for at least 28 consecutive days'. While mentioning that the outcome of the pre specified primary efficacy analysis 'did not reach significance', the CSR goes on to state, '[h]owever, for patients who received treatment according to the trial design, the Per Protocol (PP) population, Prochymal outperformed placebo 40% versus 28% (p = 0.087), essentially in line with the original protocol assumptions'. However, the PP analysis was defined in the Statistical Analysis Plan (SAP) for Study 280 as 'an exploratory analysis', with the ITT analysis being defined as the primary efficacy analysis. Furthermore, the difference between the two treatment groups as regards the analysis of the pre specified primary efficacy endpoint in the PP population was not statistically significant, and the absolute of difference of 12% between the two treatment groups is not clinically significant (based on the 20% difference specified in the sample size calculations). Furthermore, relevant TGA adopted guidelines[63] recommend the ITT population rather than the PP population as the preferred population for analysis of efficacy as it maintains the benefits of randomisation and is less subject to bias than the PP population. Consequently, for regulatory purposes it is considered that the Study 280 results of the primary efficacy analysis in the ITT population should be given more weight than the results from the exploratory analysis in the PP population.

Osiris also proffers that 'a similarly impressive effect size [to that observed in the PP for complete response for a duration of ≥ 28 days] was observed in those patients who had more steroid refractory disease, indicated by being on steroids longer than 14 days prior to study entry. In this population [ITT], 38% [24/64] of Prochymal patients had a DCR compared to only 21% [5/29] of placebo' (CSR). No statistical analysis was provided for this endpoint and the results were summarised descriptively (CSR). Furthermore, this efficacy endpoint appeared to have been defined post hoc as it could not be identified in

[63] European Medicines Agency, 'ICH Topic E 9 Statistical Principles for Clinical Trials – Step 5: Note Note for the Guidance on Statistical Principles for Clinical Trials (CPMP/ICH/363/96)', September 1998, Web, accessed 24 April 2013 <www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002928.pdf>.

the SAP for Study 280[64] and that 'the immediate goal of treatment is to stop progression of disease and to introduce a clinically meaningful response as quickly as possible', and referred to the Overall Response data as providing further support for 'the efficacy of Prochymal to enhance response in steroid refractory GvHD and provide immediate clinical benefit to the patient. An OR reflects an objective reduction of a patient's symptoms, which are severe in GvHD. Therefore, OR is itself a direct measure of clinical benefit. At Day 28, Prochymal outperformed placebo 58% to 51%. The improvement with Prochymal was statistically significant by Day 100, with 82% OR for Prochymal and 72% OR for placebo (p = 0.034)' (CSR). It was further stated that, 'the effect of Prochymal [OR at Days 28 and 56] was significantly greater when treating GvHD involving visceral organs (liver and GI tract) than skin'. However, it should be noted that the ORs for each organ at Day 28 and Day 100 in the mITT were pre specified as 'additional efficacy variables' (that is, neither primary nor secondary efficacy variables). The OR rates at Day 28 and 100 for the skin were similar in the Prochymal and placebo groups, the OR rates for the lower GIT were higher in the Prochymal group than in the placebo group at Day 28 (not statistically significant) and Day 100 (statistically significant), and the OR rates for the liver were statistically significantly higher in the Prochymal group than in the placebo group at both time points.

Osiris opines that 'once past the treatment phase and especially beyond Day 100, there are many competing risks for this severely ill patient population with a life threatening disease, which cannot be controlled within the framework of a clinical trial. These confounding factors make it difficult to evaluate survival at later times' (CSR). In an additional analysis in the mITT performed to evaluate survival while on treatment (that is, through to Day 56), survival > 56 days was higher in the Prochymal group (67.5%, n=110) than in the placebo group (59.3%, n=48) (p value not provided). The difference in survival in the overall population (liver, lower GI, skin) were primarily driven by the differences between the two treatment groups in patients with liver and lower GI involvement. In patients with liver involvement, overall survival > 56 days was 57.1% (n = 24) for Prochymal treated patients and 26.3% (n = 5) for placebo patients, for patients with lower GI involvement the respective figures were 63.5% (n = 73) and 50.8% (n = 30), and for patients with skin involvement the respective figures were 66.3% (n = 61) and 63.5% (n = 33).

Osiris stated that the results of Study 280 support 'OR at Day 28 as a clinically meaningful endpoint, especially for patients with visceral organ involvement who have very poor outcomes' (CSR). In addition, it was emphasised that OR can also serve as a surrogate endpoint by improving the chances of survival, and reference was made to 'an ad hoc analysis of survival data [which] showed the probability of survival at Day 100 was 71% for patients who attained OR and 32% for non-responders ... supporting the clinical significance of the endpoint (p <0.001, Log Rank test)'. However, at Day 28, while the OR in the Prochymal group was higher than in the placebo group the difference between the two treatment groups was not statistically significant (57.7% [94/163] and 50.6% [41/81], respectively; p = 0.224). In addition, the actual observed overall survival > 100 days did not significantly differ between the treatment groups in the mITT population (Prochymal 52.1% versus placebo 50.6%; p = 0.780). Furthermore, the OR at Day 28 was not pre specified in Study 280 as either a primary or secondary efficacy endpoint, and this endpoint appeared to be a post hoc consideration.

### Study 275 (paediatric patients)

Study 275 was a Prochymal single arm study in 59 enrolled paediatric patients aged younger than 18 years (mean ± SD age: 8.4 ± 5.97 years; range: 0.2 to 17.5 years). At

[64] The analysis was predefined.

baseline, 88.1% of enrolled patients had severe or very severe aGvHD (Grade C/D), 62.7% had received a donor unrelated transplantation, 52.5% were compatibility matched with the donor, and 61% had received prior treatment with two second line agents in addition to systemic steroids.

As discussed above, the absence of a placebo control group makes the results of this study difficult to interpret. The primary efficacy endpoint was the OR (CR + PR) at Day 28 and was observed to be 62.7% (37/59), but assessment could have taken place from day 20 to day 38. Osiris states that 'due to the refractory nature of this population, achieving any response in these patients is meaningful and largely unexpected' (CSR). However, as discussed above, the OR results at Day 28 from Study 280 in the Prochymal and placebo groups raises doubts about the validity of this statement.

The study included 4 secondary efficacy endpoints (overall response rate through Day 100, complete response rate through Day 100, response by organ involvement, and effect of continuing therapy). The OR rate through Day 100 was greater than the non-response rate in the aGvHD Grade B-D population (79.7% versus 20.3%), but conversely the CR rate through Day 100 was lower than the non-response rate (62.7% versus 37.3%). Improvement in disease status as defined by at least 1 stage reduction from baseline to Day 28 in patients with organ involvement at baseline (stage > 0) was observed in 25 of the 33 patients (75.8%) with skin GvHD, 31 of the 52 patients (59.6%) with lower GI GvHD, and 11 of the 21 patients (52.4%) with liver GvHD. Overall, 33 of 59 (55.9%) patients received > 8 Prochymal infusions and were included in the continuing therapy analysis. Of these 33 patients, 18 (54.5%) experienced an additional benefit from therapy, defined as experiencing a least a partial response from Day 28 to Day 100.

## Safety

### Overall comments

The submission included safety data on 163 patients treated with Prochymal from Study 280 (149 adults; 14 children and adolescents), 59 patients from Study 275 (children and adolescents), and 12 patients (children and adolescents) from single patient, emergency use protocols. Overall, the safety profile of Prochymal was similar in adult and paediatric populations. Consequently, the summary of safety will focus on the randomised, placebo controlled, double blind data on the 163 patients from Study 280 aged from 6 months to 70 years (inclusive).

### Study 280

The mean total cumulative Prochymal dose was 17.7 ± 6.1 x $10^6$ hMSC/kg, the mean ± SD extent of exposure was 36.0 ± 18.6 days in Prochymal group and 31.7 ± 17.5 days in the placebo group, and more than 8 infusions were received by 39.9% and 32.1% of patients in the two treatment groups, respectively. Overall, it is considered that the exposure to Prochymal for the treatment of corticosteroid resistant aGvHD is sufficient to adequately characterise the safety profile of the product for the proposed indication.

In both the treatment groups, all patients experienced at least 1 TEAE (treatment emergent adverse event) (3165 events in 163 patients in the Prochymal group, and 1376 events in the 81 patients in the placebo group). The large number of TEAEs in both treatment groups in Study 280 reflects the serious, life threatening nature of corticosteroid resistant aGvHD. Furthermore, in addition to Prochymal or placebo all patients received standard therapies (determined by individual study sites) for the disease.

The main safety concerns associated with Prochymal compared with placebo relate to the higher incidence of adverse events categorised as:

- 'infections and infestations' (primarily staphylococcal bacteraemia, pneumonia, enterococcal bacteraemia)
- 'metabolism and nutrition disorders' (primarily hypokalaemia, hyperglycaemia, hyperkalaemia, and anorexia)
- 'respiratory, thoracic and mediastinal disorders' (primarily dyspnoea, and hypoxia)
- 'psychiatric disorders' (primarily confusional state, anxiety, agitation, and insomnia)
- 'skin and subcutaneous tissue disorders (primarily rash)
- 'immune system disorders' (primarily those related to transplant rejection due to GvHD and aGvHD in intestine)
- 'cardiac disorders' (primarily those related to rate and rhythm disorders of atrial fibrillation and tachycardia) and
- the 'vascular disorder' of hypertension.

TEAEs (PT) occurring ≥ 5% more commonly in patients in the Prochymal group than in patients in the placebo group and listed from highest to lowest absolute difference between the two treatment groups were:

1. confusion and disorientation 11.6% (19.0% versus 7.4%)
2. hypertension 10.4% (17.8% versus 7.4%)
3. confusional state 9.8% (17.2% versus 7.4%)
4. anorexia 8.0% (8.0% versus 0%)
5. anxiety 7.9% (14.1% versus 6.2%) and hypokalaemia 7.9% (21.5% versus 13.6%)
6. hyperkalaemia 7.4% (12.3% versus 4.9%)
7. abdominal distension 7.3% (9.8% versus 2.5%)
8. staphylococcal bacteraemia 6.7% (12.9% versus 6.2%), tremor 6.7% (12.9% versus 6.2%), and insomnia 6.7% (14.1% versus 7.4%)
9. hyperglycaemia 6.6% (20.2% versus 13.6%)
10. mucosal inflammation 6.2% (7.4% versus 1.2%)
11. dyspnoea 6.1% (18.4% versus 12.3%)
12. dizziness 5.5% (5.5% versus 0%), pneumonia 5.5% (12.9% versus 7.4%) haematochezia 5.5% (5.5% versus 0%), and rash 5.5% (11.7% versus 6.2%) and
13. abdominal pain 5.4% (22.7% versus 17.3%).

In the safety population, the proportion of patients with SAEs leading to death (NCI CTCAE Grade 5) was higher in the Prochymal group (64.4%) than in the placebo group (54.3%). In both treatment groups, death was primarily due to 'infections and infestations' (Prochymal 25.8%; placebo 14.8%, n = 12). The only other SOC group of disorders resulting in ≥ 10% of deaths in at least one of the treatment groups was 'immune system disorders' (14.1% Prochymal, 6.2% placebo, n = 5).

Permanent discontinuation due to TEAEs was reported in 9.2% of patients in the Prochymal group and 13.6% of patients in the placebo group. There were a total of 30 TEAEs (PT) reported as leading to discontinuation in one or both of the two treatment groups. The only TEAE (PT) reported as leading to discontinuation in more than 1 patient in either of the two treatment groups was hypoxia (3 patients in the Prochymal group).

TEAEs and SAEs associated with infusional toxicity were uncommon in both treatment groups (1.8% and 2.5% of patients in the Prochymal and placebo groups, respectively). There were no TEAEs that related to ectopic tissue formation in either of the two treatment groups. TEAEs indicative of relapse of underlying disease were reported in a similar proportion of patients in the Prochymal (8.0%) and the placebo (9.9%) treatment groups. There were no marked differences between the two treatment groups as regards clinical laboratory tests, vital signs, or ECG results.

See also the CER Attachment 2 for further details of adverse events.

## First round benefit-risk assessment

### First round assessment of benefits

It is considered that the data from Study 280 have failed to satisfactorily establish the efficacy of Prochymal compared with placebo for the treatment of corticosteroid refractory aGvHD (that is, no significant treatment benefit for Prochymal has been demonstrated). In addition, the apparent benefits of Prochymal treatment in a paediatric population aged less than 18 years observed in Study 275, and in the 12 patients treated according to single patient, emergency use protocols cannot be meaningfully interpreted due to the absence of placebo control groups in these protocols.

In Study 280, the primary efficacy endpoint of complete response of ≥ 28 days duration in the overall ITT population (Grade B/C/D) was 34.7% (60/173) in the Prochymal group and 29.9% (26/87) in the placebo group; p = 0.423 (CMH [Cochran-Mantel-Haenszel] test stratified by GvHD grade at diagnosis/study entry). Similarly, there were no statistically significant differences between Prochymal and placebo for the secondary efficacy endpoints of survival at 100 days and survival at 180 days post infusion in the mITT population. The proportion of patients surviving for > 100 days in the Prochymal and placebo groups was 52.1% (85/163) and 50.6% (41/81), respectively (p = 0.780). The proportion of patients surviving for > 180 days in the Prochymal and placebo groups was 34.4% (56/163) and 42.0% (34/81), respectively (p = 0.274).

Based on expert consensus opinion, the sponsor (Osiris) considers that OR at Day 28 (following the first Prochymal infusion) is the most clinically meaningful efficacy outcome in clinical trials designed to assess the effects of treatment for aGvHD. However, OR at Day 28 was not specified as either a primary or secondary efficacy endpoint in Study 280, but was mentioned in the CSR as an additional/exploratory endpoint. Furthermore, in Study 280 there was no statistically significant difference between Prochymal and placebo in OR response at Day 28 in the overall aGvHD (Grade B/C/D) population (57.7% [94/163] and 50.6% [41/18], respectively, p = 0.224).

In Study 275, the primary efficacy outcome was OR at Day 28 in a paediatric population aged < 18 years treated with Prochymal. In this study, the OR at Day 28 in the Prochymal group was 62.7% (37/59) in the overall patient population (aGvHD Grade B/C/D). However, the clinical significance of this apparent benefit is difficult to interpret because of the absence of a placebo comparator group. It is considered that the OR at Day 28 results observed in Study 280 highlight the importance of having a placebo control comparator group when interpreting the outcomes for this endpoint (that is, no statistically significant difference between Prochymal and placebo, and a high placebo response rate observed in Study 280). Furthermore, while the protocol specified time window for the assessment of OR at Day 28 was ± 2 days, the provided result included data from patients assessed from 20 to 38 days inclusive. Consequently, the reported OR at Day 28 is potentially higher than that which would have been observed if the specified time window of ± 2 days had been used to calculate the endpoint.

The submission included a comparison between the Kaplan-Meier probabilities of survival for 180 days for patients in Study 275 (Prochymal) and a 'historical control' (external benchmark) of paediatric patients with aGvHD Grade II-IV from the CIBMTR (Centre for International Blood and Marrow Transplant Research) database. This comparison showed that the probabilities of 180 day survival for patients with maximum GvHD Grade C were 69.0% [95% CI: 64.0, 74.0] in the historical control group and 87.1% [95% CI: 70.3, 100] in the Prochymal group, and for patients with maximum GvHD Grade D the corresponding figures were 31.0% [95% CI: 26.0-36.0] in the historical control group and 56.2% [CI: 40.5-71.8] in the Prochymal group. Patients who had received Prochymal had a statistically significantly higher probability of survival compared with the historical control ($p = 0.003$). However, it should be noted that in Study 280 there was no statistically significant difference between Prochymal and placebo in the proportion of patients surviving for > 180 days.

**First round assessment of risks**

Overall, the data from Study 280 suggest that the safety profile of Prochymal is inferior to that of placebo in a number of respects. TEAEs (PT) reported in ≥ 5% more patients in the Prochymal group compared with the placebo group observed in Study 280 are listed below. The absolute difference between the two treatment groups is provided as are the results for each treatment group (Prochymal versus placebo):

- confusion and disorientation 11.6% (19.0% versus 7.4%)
- hypertension 10.4% (17.8% versus 7.4%)
- confusional state 9.8% (17.2% versus 7.4%)
- anorexia 8.0% (8.0% versus 0%)
- anxiety 7.9% (14.1% versus 6.2%) and hypokalaemia 7.9% (21.5% versus 13.6%)
- hyperkalaemia 7.4% (12.3% versus 4.9%)
- abdominal distension 7.3% (9.8% versus 2.5%)
- staphylococcal bacteraemia 6.7% (12.9% versus 6.2%), tremor 6.7% (12.9% versus 6.2%), and insomnia 6.7% (14.1% versus 7.4%)
- hyperglycaemia 6.6% (20.2% versus 13.6%)
- mucosal inflammation 6.2% (7.4% versus 1.2%)
- dyspnoea 6.1% (18.4% versus 12.3%)
- dizziness 5.5% (5.5% versus 0%), pneumonia 5.5% (12.9% versus 7.4%), haematochezia 5.5% (5.5% versus 0%), rash 5.5% (11.7% versus 6.2%) and
- abdominal pain 5.4% (22.7 versus 17.3%).

The most notable difference in TEAEs (SOC) between the two treatment groups was the higher incidence of 'psychiatric disorders' in the Prochymal group (54.6%) compared with the placebo group (37.0%). The difference between the two groups related particularly to the higher incidence of confusional state, anxiety and insomnia in the Prochymal group than in the placebo group. These are unusual and unexpected finding for this product and appear to be unrelated to possible confounders such as high dose corticosteroid use. Furthermore, Study 280 specifically excluded patients with underlying or current psychiatric conditions. In addition, there was no marked difference in baseline history of psychiatric disorders between the Prochymal group (50.3%) and the placebo group (47.1%). However, TEAEs categorised as 'psychiatric disorders' and leading to treatment discontinuation were uncommon in patients in the Prochymal (1 [0.6%] x disorientation;

1 [0.6%] x catatonia; 1 [0.6%] x delirium; 1 x [0.6%] mental state change), while no discontinuations related to this group of disorders were reported in the placebo group. In addition, there were no SAE 'psychiatric disorders' (SOC) leading to death in either treatment group. SAEs (all) 'psychiatric disorders' (PT) were reported in 3.1% patients in the Prochymal group and 2.5% of patients in the placebo group, but there were no SAEs (PT) reported as occurring in ≥ 2% of patients in the Prochymal group and ≥ 1% more commonly than in the placebo group.

Two other unexpected TEAEs occurring with higher incidences in the Prochymal group compared with the placebo group were hypertension and hyperglycaemia. There was no difference between the two treatment groups as regards baseline medical history of hypertension (46.8% versus 46.0%, Prochymal and placebo groups, respectively) or hyperglycaemia (38.2% versus 47.1%, Prochymal and placebo groups, respectively). However, a baseline medical history of diabetes mellitus (including subtypes) was notably more common in the Prochymal group (13.3%) than in the placebo group (5.7%).

The proportion of patients in the safety population who experienced at least 1 SAE was similar in the Prochymal (89.6%) and placebo groups (87.7%). However, the risk of death in the safety population was greater in the Prochymal group than in the placebo group (64.4% versus 54.3%, respectively), primarily due to 'infections and infestations' (25.8% versus 14.8%), and 'immune system disorders' (14.1% versus 6.2%). The difference between the two treatment groups (safety population) in the risk of death are consistent with the lower > 180 day survival rate in patients (mITT population) in the Prochymal group compared with patients in the placebo group (34.4% versus 42.0%, respectively; p = 0.274).

The most notable difference between the two treatment groups as regards deaths due to 'infections and infestations' (SOC) related to the HLT (High Level Term) of 'sepsis, bacteraemia, viraemia, and fungaemia NEC' which was reported in 8.0% of patients in the Prochymal group and 4.9% of patients in the placebo group. Deaths occurred notably more commonly in the Prochymal group than in the placebo group in the TEAEs (HLTs) of cytomegaloviral infections (3.1% versus 0%), adenoviral infections (3.1% versus 1.2%), aspergillus infections (2.5% versus 0%), fungal infections (1.8% versus 0%), and pseudomonal infections (1.8% versus 0%). The most notable difference between the two treatment groups as regards deaths due to 'immune system disorders' (SOC) related to the TEAE (PT) of GvHD which was reported in 9.8% of patients in the Prochymal group and 4.9% of patients in the placebo group.

SAEs (all) occurring in ≥ 2% of patients in the Prochymal group and more frequently than in the placebo group were:

- GvHD (14.7% versus 11.1%)
- gastrointestinal haemorrhage (7.4% versus 6.2%)
- sepsis (8.6% versus 7.4%)
- GvHD in intestine (6.7% versus placebo 3.7%)
- pneumonia (6.1%, versus 3.7%)
- bacteraemia (5.5% versus 3.7%)
- adenovirus infection (3.1% versus 1.2%)
- acute myeloid leukemia (3.1% versus 2.5%)
- renal failure (3.1% versus 2.5%)
- atrial fibrillation (2.5% versus 1.2%)
- staphylococcal bacteraemia (2.5% versus 1.2%)

- klebsiella bacteraemia (2.5% versus 1.2%) and
- respiratory distress (2.5% versus 1.2%).

Discontinuations due to TEAEs were reported more commonly in the placebo group than in the Prochymal group (13.6% and 9.2%, respectively). There were no marked differences between the two treatment groups as regards haematological, hepatic or renal toxicity, but cardiac disorders occurred more commonly in the Prochymal group than in the placebo group (predominantly due to tachycardia and atrial fibrillation). In Study 280, there were no notable differences between the two treatment groups as regards changes in vital signs and ECG findings over the course of the study.

There are no safety data relating to interactions between Prochymal and other medicines. There are no data on the safety of Prochymal specifically in elderly patients (aged ≥ 65 years), or separately in males and females. There are no safety data relating to rebound or withdrawal effects following discontinuation of Prochymal.

### First round assessment benefit-risk balance

The benefit-risk balance for Prochymal for the proposed usage is considered to be unfavourable. The submitted data have failed to demonstrate a meaningful clinical benefit in patients with corticosteroid resistant aGvHD treated with Prochymal compared with placebo, and the risks of treatment with Prochymal for the proposed usage are considered to be greater than those of placebo.

### First round recommendation regarding authorisation

It is recommended that the application to register Prochymal for the proposed indication be rejected on the grounds of inadequate demonstration of efficacy. The reasons for this recommendation are as follows:

1. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo for the primary efficacy endpoint of complete response of ≥ 28 days duration in the overall ITT population (aGvHD Grade B/C/D): that is, 34.7% (60/173) and 29.9% (26/87), respectively, p = 0.423
2. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo for the secondary efficacy endpoint of overall survival > 100 days in the overall mITT population (aGvHD Grade B/C/D): that is, 52.1% (85/163) and 50.6% (41/81), respectively, p = 0.780
3. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo in the secondary efficacy endpoint of overall survival > 180 days in the overall mITT population (aGvHD Grade B/C/D): that is, 34.4% (56/163) and 42.0% (34/81), respectively, p = 0.274
4. The lack of a placebo control group in Study 275 (supportive study) making it difficult to interpret the clinical significance of the observed overall response rate at day 28 of 62.7% (37/59) in a paediatric population aged < 18 years (i.e., the primary efficacy endpoint). The importance of including a placebo control group when assessing the efficacy of Prochymal is highlighted by the results from Study 280 for overall response at Day 28 which failed to show a statistically significant difference between the two treatment groups: that is, 57.7% (94/163) in the Prochymal group compared with 50.6% (41/81) in the placebo group, p = 0.224.

## List of questions

### Pharmacokinetics

No questions.

### Pharmacodynamics

No questions.

### Efficacy

In Study 275, the study specified that the primary efficacy endpoint of OR was to be assessed at Day 28 ± 2 days. However, the provided results for the OR at Day 28 included assessments undertaken between study Days 20 and 38.

1. Please explain why the submitted analysis was undertaken on patients assessed outside Day 28 ± 2 days.
2. Please justify how the provided assessment of OR at Day 28 (day 20 to day 38) can be considered to be a surrogate for assessment of OR at Day 28 ± 2 days.
3. Please provide an analysis of only those patients who had an assessment for OR at Day 28 ± 2 days.

### Safety

No questions.

## Second round evaluation of clinical data

### Overview of the second round data

The sponsor's Section 31 response, dated 30 April 2012, included responses to the clinical questions raised by the TGA following the first round evaluation of the submission. Clinical comments on the sponsor's response are provided below. In addition, the TGA quality evaluator has recommended that clinical advice be provided on certain matters referred to in the sponsor's response to first round evaluation quality questions. This advice has been provided below. The first and second round clinical evaluation reports have been prepared by the same clinical evaluator, and this evaluator has also provided clinical advice on the matters identified by the TGA quality evaluator.

### Sponsor's response to clinical questions and evaluator's comments

#### *Efficacy (question 1)*

In Study 275, the study specified that the primary efficacy endpoint of OR was to be assessed at Day 28 ± 2 days. However, the provided results for the OR at Day 28 included assessments undertaken between study Days 20 and 38:

- Please explain why the submitted analysis was undertaken on patients assessed outside Day 28 ± 2 days
- Please justify how the provided assessment of OR at Day 28 (Day 20 to Day 38) can be considered to be a surrogate for assessment of OR at Day 28 ± 2 days
- Please provide an analysis of only those patients who had an assessment for OR at Day 28 ± 2 days.

### *Sponsor's (Osiris) response*

In Study 275, the Prochymal treatment regimen is 8 infusions, each infusion given 3-4 days apart, for a treatment period of about 24-25 days. In the original version of the study, dated 4 June 2007, the first GvHD assessment was set at Day 32 ± 2 days, one week after the 8th infusion, since the practice of most clinical sites is to assess GvHD status on a weekly basis. There was then a growing consensus in the GvHD community that OR at Day 28 is the optimal endpoint for assessing treatment effect in acute GvHD. This consensus was subsequently endorsed by the experts of a FDA/CIBMTR workshop on clinical endpoints in acute GvHD. As a result, the study was amended to adjust the GvHD assessment from Day 32 ± 2 days to Day 28 ± 2 days (Amendment 1, 14 March 2008).

Out of 59 patients, 36 patients completed their GvHD assessment according to protocol (Day 28 ± 2 or Day 32 ± 2) (Table 3).

**Table 3: GvHD assessment data**

| GvHD Assessment (days) | Number of Patients | OR | Comments |
|---|---|---|---|
| 32 ± 2 days | 8 | 7/8 (87.5%) | Original protocol |
| 28 ± 2 days | 28 | 19/28 (67.9%) | Protocol Amendment 1 onwards |
| 24-25 days | 12 | 9/12 (75.0%) | Site performed assessment at last infusion of the initial treatment schedule visit |
| 21-38 days | 5 | 2/5 (40.0%) | These patients completed a full GvHD assessment out of protocol window |
| None (death prior to Day 28) | 6 | n/a (non-responders) | Patients died and an end-of-study evaluation page was completed |
| Total | 59 | 37/59 (62.7%) | |

For 42 patients (71%), the GvHD assessments were performed according to protocol. An additional 12 patients had the GvHD assessment performed immediately after the last Prochymal infusion (Day 24-25). Of importance, all of these patients (54/59, 92%) received the full treatment course of 8 infusions or died prior to reaching Day 28. The treatment compliance for these 54 patients is 100%. The GvHD status for these patients represents a good assessment of the effect of a full course of Prochymal infusions.

A total of 5 patients underwent the GvHD assessment in a 21 to 38 day window. These assessments were completed outside the recommended protocol window for a variety of reasons, some related to the compromised nature of these very sick patients and some related to the physician's request for continued therapy. Of these five patients, one patient died at Day 21 after 6 infusions. The patient had completed a full GvHD assessment the day of death and was included in the analysis. The patient was a non-responder. Another patient completed the GvHD assessment at Day 22 after 7 infusions. The patient was a partial responder and the physician requested continued therapy. A third patient had the GvHD status assessed at Day 23 after 8 infusions, was a partial responder and also continued therapy. A fourth patient had the GvHD assessment at Day 31 after 8 infusions. The assessment was conducted on a Monday (Day 31) and according to the window should have been done the Friday before (Day 28). A fifth patient was evaluated after 8 infusions at 38 days, and reasons for the delay are unknown.

### *Clinical evaluator's comment*

The sponsor's response is satisfactory and has clarified the assessment of Prochymal treatment in supportive Study 275. The primary efficacy endpoint in this protocol was OR (CR + PR) at Day 28. The Day 28 assessment was based on response data reported between Days 20 and 38 from all 59 patients, and included patients who died before day 28 as non-responders. The OR rate in patients with GvHD Grade B/C/D in the primary efficacy analysis was 62.7% (37/59).

The sponsor's Section 31 additional data show that in patients analysed at 28 ± 2 days the OR was 67.9% (19/28), and in patients analysed at 32 ± 2 days the OR was 87.5% (7/8). However, assessments at these time points were aimed at determining whether further treatment was indicated and excluded patients who had died prior to assessment. Therefore, the OR analyses at these time points are not comparable to the primary efficacy analysis of the OR in which patients who had died were included in the assessment as non-responders. The primary efficacy endpoint analysis is considered to be a reasonable clinical measure of overall response to treatment as it includes relevant response data from all 59 treated patients. However, it is noted that if response is assessed at Day 28 ± 2, and includes patients who died prior to Day 28 as non-responders, then the OR is 55.9% (19/34) which is lower than the OR in the primary analysis (62.7%; 37/59). Overall, the additional data submitted by the sponsor do not alter the first round assessment of the benefits or the benefit-risk balance of Prochymal for the proposed indication.

### Requested clinical advice on responses to first round quality assessment

#### *Toxicity of infused dead cells*

The TGA quality evaluator has requested clinical advice on the whether the toxicity of infused dead cells has been sufficiently addressed. The TGA quality evaluator notes that:

> *'it is not stated in the dossier, but within the undifferentiated MSC population (≥ 95% of the cells) there may be MSC subpopulation types (antigenically defined or otherwise). It is evident the current literature has not fully defined the MSC subpopulations or provided standardised criteria for their characterisation. Provided there is an assurance of a consistent and reproducible manufacturing process, there is unlikely to be a basis on quality grounds for requesting a further definition of Prochymal in relation to its MSC population subtypes. With respect to other viable cell-based impurities, these may include viable CD45+ cells and other undefined CD45-/CD105-/CD166- cells (<5%), which may be retained through the process of mononuclear cell isolation, plastic culture plate adherence, trypsinisation and cell washes over 5 passages in culture'.*

#### *Clinical evaluator's comment*

The first round risks of treatment with Prochymal are summarised. This assessment was primarily based on the placebo controlled data from Study 280. Overall, it is considered that the risks of treatment with Prochymal have been adequately characterised in Study 280. The patient numbers in Study 280 were small in both the Prochymal (n = 163) and placebo (n = 81) treatment groups, but this is not unexpected given that Prochymal is a designated orphan drug. If the Prochymal formulation used in Study 280 notably differs from that being proposed for registration, then the risks of treatment with the proposed formulation might differ from those observed in Study 280.

## Second round benefit-risk assessment

### Second round assessment of benefits

After consideration of the responses to clinical questions, the wording of the benefits of Prochymal in the proposed usage have been amended to delete speculation on the effect of potential differences in response between the primary efficacy analysis and the efficacy analysis at 28 ± 2 days. The second round assessment of benefits follows.

It is considered that the data from Study 280 have failed to satisfactorily establish the efficacy of Prochymal compared with placebo for the treatment of corticosteroid refractory aGvHD (that is, no significant treatment benefit for Prochymal has been

demonstrated). In addition, the apparent benefits of Prochymal treatment in a paediatric population aged < 18 years observed in Study 275, and in the 12 patients treated according to single patient, emergency use protocols cannot be meaningfully interpreted due to the absence of placebo control groups in these protocols.

In Study 280, the primary efficacy endpoint of complete response of ≥ 28 days duration in the overall ITT population (Grade B/C/D) was 34.7% (60/173) in the Prochymal group and 29.9% (26/87) in the placebo group; p = 0.423 (CMH test stratified by GvHD grade at diagnosis/study entry). Similarly, there were no statistically significant differences between Prochymal and placebo for the secondary efficacy endpoints of survival at 100 days and survival at 180 days post infusion in the mITT population. The proportion of patients surviving for > 100 days in the Prochymal and placebo groups was 52.1% (85/163) and 50.6% (41/81), respectively (p = 0.780). The proportion of patients surviving for > 180 days in the Prochymal and placebo groups was 34.4% (56/163) and 42.0% (34/81), respectively (p = 0.274).

Based on expert consensus opinion, the sponsor considers that OR at Day 28 (following the first Prochymal infusion) is the most clinically meaningful efficacy outcome in clinical trials designed to assess the effects of treatment for aGvHD. However, OR at Day 28 was not specified as either a primary or secondary efficacy endpoint in Study 280, but was mentioned in the CSR as an additional/exploratory endpoint. Furthermore, in Study 280 there was no statistically significant difference between Prochymal and placebo in OR response at Day 28 in the overall aGvHD (Grade B/C/D) population (57.7% [94/163] and 50.6% [41/18], respectively, p = 0.224).

In Study 275, the primary efficacy outcome was OR at Day 28 in a paediatric population aged < 18 years treated with Prochymal. This assessment was based on outcomes over a broad window of from 20 to 38 days. In this protocol, the OR at 'Day 28' in the Prochymal group was 62.7% (37/59) in the overall patient population (aGvHD B/C/D). However, the clinical significance of this apparent benefit is difficult to interpret because of the absence of a placebo comparator group. It is considered that the OR at Day 28 results observed in Study 280 highlight the importance of having a placebo control comparator group when interpreting the outcomes for this endpoint (that is, no statistically significant difference between Prochymal and placebo, and a high placebo response rate observed in Study 280).

The submission included a comparison between the Kaplan-Meier probabilities of survival for 180 days for patients in Study 275 (Prochymal) and a historical control (external benchmark) of paediatric patients with aGvHD Grade II-IV from the CIBMTR database. This comparison showed that the probabilities of 180 day survival for patients with maximum GvHD Grade C were 69.0% [95% CI: 64.0, 74.0] in the historical control group and 87.1% [95% CI: 70.3, 100] in the Prochymal group, and for patients with maximum GvHD Grade D the corresponding figures were 31.0% [95% CI: 26.0-36.0] in the historical control group and 56.2% [CI: 40.5-71.8] in the Prochymal group. Patients who had received Prochymal had a statistically significantly higher probability of survival compared with the historical control (p = 0.003). However, it should be noted that in Study 280 there was no statistically significant difference between Prochymal and placebo in the proportion of patients surviving for > 180 days.

### Second round assessment of risks

After consideration of the responses to clinical questions, the risks of Prochymal in the proposed usage are unchanged from those identified in the first round assessment.

### Second round assessment of benefit-risk balance

After consideration of the responses to clinical questions, the benefit risk-balance of Prochymal in the proposed usage is unchanged from those identified in the first round assessment. The benefit-risk benefit remains unfavourable for the reasons provided in the first round assessment.

### Second round recommendation regarding authorisation

It is recommended that the application to register Prochymal for the proposed indication be rejected on the grounds of inadequate demonstration of efficacy. The reasons for this recommendation are as follows:

1. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo for the primary efficacy endpoint of complete response of ≥ 28 days duration in the overall ITT population (aGvHD Grade B/C/D): that is, 34.7% (60/173) and 29.9% (26/87), respectively, p = 0.423.
2. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo for the secondary efficacy endpoint of overall survival > 100 days in the overall mITT population (aGvHD Grade B/C/D): that is, 52.1% (85/163) and 50.6% (41/81), respectively, p = 0.780.
3. The failure of Study 280 (pivotal study) to demonstrate a statistically significant difference between Prochymal and placebo in the secondary efficacy endpoint of overall survival > 180 days in the overall mITT population (aGvHD Grade B/C/D): that is, 34.4% (56/163) and 42.0% (34/81), respectively, p = 0.274.
4. The lack of a placebo control group in Study 275 (supportive study) making it difficult to interpret the clinical significance of the observed overall response rate at 'day 28' of 62.7% (37/59) in a paediatric population aged < 18 years (that is, the primary efficacy endpoint). The importance of including a placebo control group when assessing the efficacy of Prochymal is highlighted by the results from Study 280 for overall response at day 28 which failed to show a statistically significant difference between the two treatment groups: that is, 57.7% (94/163) in the Prochymal group compared with 50.6% (41/81) in the placebo group, p = 0.224.

# V. Pharmacovigilance findings

## Risk management plan

The sponsor submitted a Risk Management Plan that was reviewed by the TGA's Office of Product Review (OPR).

### Safety specification

The sponsor provided a summary of Ongoing Safety Concerns which are shown at Table 4.

**Table 4: Ongoing Safety Concerns for Prochymal**

| | |
|---|---|
| **Important identified risks** | None |
| **Important potential risks** | Infusion-associated reactions |
| | Ectopic tissue formation |
| | Infection |
| | Hypersensitivity to porcine/bovine excipients |
| **Important missing information** | Use in patients who received HSCT for solid tumour |
| | Use in patients with pre-existing neoplasms or active relapse of underlying disease |
| | Use in patients with renal dysfunction |
| | Use in pregnancy |

***OPR reviewer's comment***

Pursuant to the evaluation of the nonclinical and clinical aspects of the Safety Specification (SS), it is recommended that the additional safety concerns identified by the clinical reviewer (psychiatric reactions, hypertension, hyperglycaemia) be added to the list of important identified risks.

**Pharmacovigilance plan**

***Proposed pharmacovigilance activities***

Proposed pharmacovigilance activities are:

- Routine pharmacovigilance
- Post authorisation study. Study 295 is an open label study proposed to follow 65 paediatric patients aged 6 months to 17 years for 3 years after starting Prochymal. This study has not started and
- Follow up of patients in other studies. Study 275 is a paediatric study in the US and Canada (anticipated enrolment up to 50 patients per year; enrolment so far 59 patients), and Study 276 is an adult study in the US (anticipated enrolment up to 120 patients per year; enrolment so far 3 patients). Both are open label, 'expanded access' programs which follow enrolled patients for up to 100 days.

All three studies enrol patients with GvHD who fail to respond to steroids.

***OPR reviewer's comments in regard to the pharmacovigilance plan and the appropriateness of milestones***

Of the important potential risks identified by the sponsor, infusion related and hypersensitivity reactions occur at the time of administration of the medicine, and are likely to have been adequately captured in the clinical trial program. The other two identified potential risks, ectopic tissue formation and infection, may occur some time after treatment. The proposed long term/extension studies appear inadequate to address these two potential risks.

To study the risk of ectopic tissue formation, it is expected that a study of larger than 65 patients is required. Regarding the risk of infection, a duration of 100 days may be adequate to identify relevant infections. However, it is already known that the background risk of infection in patients undergoing HSCT is already high, and so any meaningful safety study in this area should include a control group.

The sponsor has not proposed any strategy for addressing the identified important missing information. In order to identify patients receiving this medicine outside of the clinical trial inclusion criteria, it would seem necessary to establish a register of patients receiving Prochymal. This should be feasible, given the small population who will receive

the medicine, and the limited number of centres at which such treatment is performed – estimated by the sponsor to be no more than 15 centres in Australia.

In conclusion, the sponsor's proposed pharmacovigilance activities – Studies 275, 276, and 295 – do not seem to address the potential risks identified by the sponsor.

### Evaluation of the need for risk minimisation activities

#### *Sponsor's conclusion in regard to the need for risk minimisation activities*

The sponsor does not provide any specific conclusion regarding the need for risk minimisation activities, merely listing the proposed activities (see 'Risk minimisation activities', below).

#### *Potential for medication errors*

Given the setting in which the medication is supplied: prepared for single patient use, and given in a small number of highly specialised tertiary care centres, the potential for medication error is low.

#### *Toxicity in overdose*

Given the setting in which the medication is supplied, the potential for overdose is low. The PI states that there have been no reports of overdose, and that the maximum tolerated dose in humans has not been established.

*Risk minimisation activities*

Apart from appropriate wording in the PI, the only risk minimisation activity explicitly proposed by the sponsor is around infusion associated reactions. The PI recommends premedication with hydrocortisone and diphenhydramine, controlled rate of infusion, infusion under supervision, and monitoring of oxygen saturation during infusion. This appears appropriate.

### Summary of recommendations

The OPR provides these recommendations in the context that the submitted RMP is supportive to the application; the implementation of a RMP satisfactory to the TGA is imposed as a condition of registration; and the submitted EU-RMP is applicable without modification in Australia unless so qualified:

1. Full tumourogenicity studies should be undertaken.
2. Psychiatric reactions, hypertension, and hyperglycaemia should be added to the list of important identified risks in the SS.
3. A register of patients receiving Prochymal in Australia should be established.
4. The sponsor should specifically address how the risk of ectopic tissue formation (potentially a long term complication) is to be assessed.
5. The sponsor should include a control group in a post marketing study intended to gather safety information, such as the risk of infection.

# VI. Overall conclusion and risk/benefit assessment

The submission was summarised in the following Delegate's overview and recommendations:

## Quality

Prochymal is *ex vivo* cultured hMSCs derived from the bone marrow of unrelated and HLA unmatched healthy adult donors.

The BMA is not pooled so that a finished product contains cells from only one donor. The sponsor has set up a program for traceability of donor qualification and testing information. The sponsor obtains BMA as raw material from a contract manufacturer who is responsible for their own proprietary procedures involving qualification, screening, testing and procurement of BMA, as well any contractual arrangements or remuneration of the donors.

The quality evaluators consider the donor eligibility, screening and testing requirements to be acceptable and consistent with regulatory guidelines.[65]

The DS manufacture involves maintaining aseptic conditions in a functionally closed system. There are no specific bioburden reduction steps or terminal sterilisation processes. The manufacture does not involve using human embryo, human embryonic stem cell, other materials sourced from a human embryo or human embryonic stem cell. All other materials (biological and non-biological) used in manufacture are reported to be appropriately sourced and tested with the exception of FBS which was sourced from the US for the currently held stock of the product.

**For future batches FBS and any release in Australia, it is considered that it be sourced from zero risk countries (Australia and New Zealand).**

The criteria used for characterising hMSCs are reported to be consistent with the International Society for Cellular Therapies minimum criteria for defining multipotent mesenchymal stromal cells. The main characterisation of cell phenotype and identity is based on three cell surface markers (CD45, CD105 and CD166).

The characterisation of potency is based on immunomodulative and anti-inflammatory properties of hMSCs. The sponsor developed an assay panel consisting of TNF RI, IL-2Rα and phenotype.

**The sponsor has committed to implement characterisation of MHC I and MHC II for the DS lot release testing.**

The submitted data support a proposed shelf life of 48 months for the DS stored at ≤ -135°C in the liquid nitrogen vapour phase.

**However, the final study reports for these studies are pending.**

The DP is packaged in cryogenic freezing containers in a final volume of approximately 15mL. The labelling for DP is approximately  hMSCs formulated in Plasma-Lyte A containing HSA and DMSO for storage at LN2 vapour phase temperature (≤ -135°C) until thawed and reconstituted before administration. Once reconstituted, Prochymal must be kept at room temperature and infused within 5 h after thawing.

Impurities associated with the manufacture of the DP are stated to be residual BSA, residual heparin, residual trypsin and particulates inherent to the manufacturing process. Impurities such as viral and other adventitious agents are controlled at the DS manufacturing stage. Testing for residual heparin is not performed as the estimated residual level in the DP is lower than the limits of detection and is not considered to have safety impact. Residual testing for BSA and trypsin is included in the specifications. Notably, dead cells, cell debris, and non hMSCs were not stated as DP impurities.

[65] Therapeutic Goods Administration, 'Australian Regulatory Guidelines for Biologicals Appendix 4: Guidance on donor selection, testing and minimising infectious disease transmission via therapeutic goods that are human blood and blood components, human tissues and human cellular therapy products', August 2011

**The potential for unidentified CD45-/CD105-/CD166 cells to be present as low level impurities in the DP (< 1% to 5% of the DP) was not fully addressed by the sponsor.**

The excipients are also considered appropriately controlled. Overall the DS/DP specifications are considered by the quality evaluators to be acceptable and the manufacturing process as sufficiently controlled.

However, a number of manufacturing process changes were implemented in 2009 and were subsequent to the completion of the process validations for the DP. These included changes to cell seeding parameters.

Certain testing was included in DS specifications, but not for the DP specifications. The DS is subjected to a further 3 passages in culture and a test for chromosomal aberrations at the later DP stage may be appropriate given the known potential for hMSC transformation in *ex vivo* cultures.

**The sponsor has agreed to institute a monitoring plan to test DP and gather data to demonstrate that DP and DS are consistent. This commitment is supported but the plan has not been submitted or reviewed.**

**The sponsor has also committed to analysing the batch analysis data for the DS and DP and will revise and tighten all applicable lot release specifications as appropriate prior to release of any newly manufactured DP or DS lots as suggested by the quality evaluators.**

There are no outstanding quality issues in relation to bacterial endotoxin safety, pathogen safety (including viral, prion and mycoplasma). With the exception of sterility and endotoxin DP specification test requirements, there is no other pharmacopoeial test or final product monograph applicable to the DP. The TGA's Office of Laboratory & Scientific Services has advised that there are no batch releases testing requirements for this type of biological medicine.

The current product labels are non-compliant with the regulatory requirements and will require changes or exemptions.

> A number of conditions of registration have been proposed by the quality evaluators in relations to the matters identified above.
>
> GMP clearance for all manufacturing sites is pending.

The PSC advice was sought for three outstanding issues in its 147th meeting. The PSC organised an external expert to provide advice in relation to this product, following which further information was sought from the sponsor. The quality evaluators report follows.

***Inadequacy of the sterility testing of the in process wash solutions***

PSC considered that a 7 day incubation period would be acceptable if there are data to establish equivalence of the two testing methods. In the absence of data demonstrating equivalence of the two methods, the sponsor should be asked to revise their validation study to show that the detection systems are equivalent.

This is acceptable.

**The proposed new USP/EU 14 day sterility test must be validated and approved by TGA before release of product in Australia.**

***Adequacy of the panel of assays (cell viability, TNF R1 and IL-2Rα) for assessing Prochymal potency during storage***

PSC advised that TNF-R1 was not an appropriate test for assessing cell degradation or stability of the drug product. The PSC advised that cell viability assay is currently the best indicator of product degradation.

***Changes introduced in 2009 to streamline the manufacturing process for the commercial DP were not appropriately validated or justified***

The changes included removal of cell count information and microscopic observation of morphology and growth characteristics at thaw, Passage 4, Passage 5. There is an additional concern that due to the insufficient characterisation of the change it was difficult to compare the quality of the DP manufactured under the commercial process compared to the product tested in Phase 3 clinical trials. The data submitted was deemed insufficient to justify the change as it could represent a reduction in process control. The PSC advised that the changes made to the drug product manufacturing process are unacceptable. The PSC was concerned that the removal of certain steps may introduce even greater variability, and therefore agreed that the manufacturer has not satisfactorily demonstrated process control. The PSC concluded that given the potentially greater variation in population doublings, the sponsor should be asked to provide fuller/broader characterisation of the cells in relation to the process changes. Following this Osiris has proposed to reinstate a number of process steps to ensure that the biological characteristics and properties of the DP are consistent. In addition, the sponsor has agreed to develop a protocol to perform additional characterisation of the cells manufactured.

**The protocol will be provided to the TGA for review and comment prior to its execution.**

The quality evaluators advise that reinstatement of the process steps outlined above by the sponsor is deemed sufficient to control the current manufacturing process. Additional characterisation, as proposed, would only be required if the sponsor intends to validate the proposed streamlined process in the future. There is no objection to the approval of Prochymal (remestemcel-L, *ex vivo* adult hMSC, cell suspension, 100 x $10^6$ cells per 15 mL bag) on quality grounds. However, the following issues should be noted:

**A number of changes have been introduced to the manufacturing process for Prochymal in response to issues raised by TGA. On this basis, the manufacturing process utilised for generation of currently held stock of Prochymal is not in compliance with TGA's requirements and should not be released in Australia.**

## Nonclinical

An inherent limitation in the nonclinical testing programme is the compulsion to use homologous cells in animal models. Also no efficacy studies for aGvHD could be modelled. These limitations are understandable and are considered acceptable.

However, the nonclinical evaluator does not support registration of the product because:

> *'the evidence set forth in the submitted dossier is limited in depth and breadth in terms of mechanism of action, efficacy and safety studies. In many of the presented studies, information regarding specific culture conditions such as, passage numbers, homogeneity of culture, in vitro differentiation potential and cytokine activity profile of cells were not provided.'*

In the opinion of the nonclinical evaluators the presentation of these parameters is essential for accurate understanding and interpretation of data provided.

In particular, evidence for *in vivo* tissue homing was found to be ambiguous. The presented data did not provide strong evidence for increased engraftment of MSCs to tissues damaged by irradiation, and thus by extension, tissues that are damaged by GvHD. The study data did not allow for *in situ* identification of transplanted cells as MSCs or differentiated cells.

The data included in the dossier on tissue protection and repair did not on the whole utilise MSCs or use adequate analysis when MSCs were tested.

Repeat dose toxicity was only performed in one rodent species, no non rodent repeat dose studies were performed as required by relevant ICH guidelines. No toxicity studies were conducted in young animals. In consideration of published literature indicating involvement of MSCs in tumour growth and/or propagation, the nonclinical evaluator believes that further carcinogenicity/tumourigenicity studies were warranted.

No reproductive studies were performed with Prochymal, due to the poor survival prognosis of patients. A pregnancy category (B3) has been proposed by the sponsor.

## Clinical

### Efficacy

The clinical dossier consisted of 2 clinical efficacy studies and reports of individual emergency use in 12 children. There were no PK, PD or biodistribution studies norwere there formal dose finding studies. There are no efficacy/safety data addressing rebound or withdrawal effects following discontinuation of Prochymal.

Of the two clinical studies supporting this submission, Study 280 was a randomised, controlled trial in adults and children, while Study 275 was uncontrolled treatment in children.

#### *Study 280*

This was study of Prochymal for the treatment of aGvHD patients (Grade B/C/D) who had failed to respond to steroid treatment. The study design was multinational (including Australia), multicentre, randomised, placebo controlled, double blind. The mean (SD) age of patients was 42.6 ± 17.2 years. A subgroup of paediatric patients consisted of 28 children and adolescents.

The patients (n = 260) were randomised (2:1) to Prochymal and placebo groups. The drug dose and administration ($2x10^6$ hMSC/kg IV twice a week for 4 weeks) was consistent with the regime being proposed for approval. All patients were to receive 8 infusions as initial treatment by study Day 28. Infusions were administered at least 3 days apart. On Day 32, the patients were assessed were continuation of treatment. For a Complete Response (CR), no additional study drug was given. For No Response (NR), no additional drug was administered. For Partial Response (PR) or Mixed Response (MR), the study drugs were continued once per week for a further 4 weeks. The study permitted concomitant GvHD therapies in accordance with site specific institutional policies. The severity of aGvHD was assessed by a physician using IBMTR Grading.

*Primary efficacy outcome: CR of 28 days*

The primary efficacy variable evaluated was CR (resolution of acute GvHD in all involved organs) of ≥ 28 days within 100 days post first infusion. The results are shown in Table 5 using ITT population.

**Table 5: Complete response of ≥ 28 days duration by treatment group and by GvHD grade (ITT) (Study 280)**

| | GVHD (All grade B/C/D) | | | GVHD Grade B | | GVHD Grade C/D | |
|---|---|---|---|---|---|---|---|
| | Prochymal (n=173) | Placebo (n=87) | p-value | Prochymal (n=38) | Placebo (n=23) | Prochymal (n=135) | Placebo (n=64) |
| Responder | 34.7% (n=60) | 29.9% (n=26) | p = 0.42 | 39.5% (n=15) | 30.4% (n=7) | 33.3% (n=45) | 29.7% (n=19) |
| Non-Responder | 65.3% (n=113) | 70.1% (n=61) | | 60.5% (n=23) | 69.6% (n=16) | 66.7% (n=90) | 70.3% (n=45) |

Responder (= CR) is defined as a subject with a complete response of 28 days or more. Non-responder includes patients who have no recorded CR, or a CR lasting less than 28 days.

Thus, the CR response rate was 34.7% versus 29.9% for Prochymal and placebo groups respectively and the treatment difference was not statistically significant (p = 0.42). The response rate using PP population was 11.2% and 4.8%, respectively (p=0.087). Similarly, the treatments differences between the two groups by aGvHD grade were also not statistically significant. The median time to CR was 21 days (95%CI 16 to 28 days) in Prochymal group compared to 25 days (95%CI 17 to 32 days) in placebo group (p = 0.26). The results for some secondary/additional efficacy outcomes are below.

*OR at Day 28*

The OR rate (CR + PR) at Day 28 (± 4 days) using mITT population was 57.7% (94/163) versus 50.6% (41/81) for Prochymal versus placebo groups respectively (p = 0.22).

*OR at Day 100*

The OR (all grades) at Day 100 was 82% versus 72% for Prochymal and placebo groups respectively (p = 0.03). The organ specific GvHD OR rates at Days 28 and 100 were as shown in Table 6.

**Table 6: Overall response (Study 280)**

| | Prochymal | Placebo | Absolute Effect Size (Prochymal-Placebo) | P-Value* |
|---|---|---|---|---|
| **OR at Day 28** | | | | |
| Liver | 55% (23/42) | 21% (4/19) | +34% | 0.025 |
| Lower GI | 57% (65/115) | 42% (25/59) | +15% | 0.081 |
| Skin | 58% (53/92) | 56% (29/52) | +2% | 0.862 |
| **OR by Day 100** | | | | |
| Liver | 76% (32/42) | 47% (9/19) | +29% | 0.039 |
| Lower GI | 82% (94/115) | 64% (38/59) | +18% | 0.015 |
| Skin | 78% (72/92) | 77% (40/52) | +1% | 0.838 |

* Fisher's Exact test.

*Overall survival at 100/180 days post first infusion*

The percentage of patients surviving for greater than 100 days post first infusion was 52.1% (85/163) versus 50.6% (41/81) in Prochymal and placebo groups respectively (p = 0.78). The percentage of patients surviving for > 180 days post first infusion was 34.4% (56/163) versus 42.0% (34/81) in Prochymal and placebo groups respectively (p = 0.27).

The median survival time in both treatment groups was 115 days (p = 0.52). Overall, there were no marked differences between the two treatment groups with respect to cumulative steroid use.

*Paediatric subgroup in Study 280*

The paediatric subpopulation enrolled in Study 280 consisted of both male and female patients, aged between 6 months and < 18 years, with steroid refractory aGvHD (Grade B/C/D) secondary to allogeneic HSCT (bone marrow, peripheral blood stem cells, or cord blood cells) or DLI. Overall, 28 paediatric patients were included (14 in each treatment group) and 25/28 (89.3%) completed the study (13 and 12 in Prochymal and placebo groups, respectively). In the Prochymal group, the mean (SD) age was 6.5 (3.8) years compared to 9.3 (5.7) years in the placebo group. The results were as shown in Table 7.

**Table 7: Efficacy in the paediatric subgroup (All Grades B/C/D) – ITT population (Study 280)**

| Efficacy Endpoint | Prochymal (n=14) | Placebo (n=14) | Treatment difference | p-value |
|---|---|---|---|---|
| CR ≥ 28 days duration | 64.3% (9/14) | 42.9% (6/14) | **21.4%** | p=0.274. |
| Survival at 100 days | 78.6% (11/14) | 50.0% (7/14) | **28.6%** | p=0.129 |
| Survival at 180 days | 64.3% (9/14) | 50.0% (7/14) | **14.2%** | p=0.461. |
| CR through Day 100 | 71.4% (10/14) | 50.0% (7/14) | **21.4%** | p=0.261 |
| OR at Day 28 | 64.3% (9/14) | 35.7% (5/14) | **28.6%** | p=0.139 |
| OR through Day 100 | 85.7% (12/14) | 57.1% (8/14) | **21.4%** | p=0.107 |

All efficacy outcome numerically favoured Prochymal over placebo in the paediatric subgroup, but the treatment differences were statistically not significant.

### *Study 275*

This supportive uncontrolled study was designed to use Prochymal as a rescue agent in treatment refractory (steroids and other second line immune modulating agents) aGvHD. A total of 59 paediatric patients from multiple sites in 6 countries (US, Canada, UK, Italy, Finland, and New Zealand) participated in this trial. The age range was 2 months to 17 years (mean (SD) 8.4 (6) years) with Grades B/C/D aGvHD secondary to allogeneic HSCT or DLI who had failed to respond to steroid treatment and other therapies. Concomitant therapies at the investigator's discretion and in accordance with site specific institutional policies were allowed.

The designated primary efficacy endpoint was OR (CR + PR) at Day 28 (assessed between study Days 20 and 38) and was as shown in Table 8.

**Table 8: Overall response (CR + PR) at Day 28 (N = 59) (Study 275)**

| | All GVHD grades B/C/D (n=59) | GVHD Grade B (n=7) | GVHD Grade C (n=17) | GVHD Grade D (n=35) |
|---|---|---|---|---|
| Responder | 37 (62.7%) | 5 (71.4%) | 13 (76.3%) | 19 (54.3%) |
| Non-Responder | 22 (37.3%) | 2 (28.6%) | 4 (23.5%) | 16 (45.7%) |

The OR rate (all grades) was higher in patients with Grades B/C disease. In patients with grade D disease (the most severe category), 54% patients responded compared to 46% non-responders.

A number of secondary efficacy endpoints were also examined as follows:

*Overall Response (CR + PR) rate through to Day 100:*

47/59 (79.7%) responders and 12/59 (20.3%) non responders (all grades). The corresponding results (responders versus non responders) by severity grade were as follows:

Grade B: 7/7 (100%) versus nil

Grade C: 15/17 (88.2%) versus 2/17 (11.6%)

Grade D: 25/35 (71.4%) versus 10/35 (28.6%)

*Complete response (CR) rate through to Day 100:*

22/59 (37.3%) responders and 37/59 (62.7%) non responders (all grades). The corresponding results (responders versus non responders) by disease severity grade were as follows:

Grade B: 5/7 (71.4%) versus 2/7 (28.6%)

Grade C: 8/17 (47.7%) versus 9/17 (52.9%)

Grade D: 9/35 (25.7%) versus 26 (74.3%)

*Response by organ involvement*

*Skin GvHD*

Overall, 25 (75.8%) of the 33 patients with skin GvHD at baseline (stage > 0) had improvement in their skin disease at Day 28, with complete resolution occurring in 13/33 (39.4%) patients. Four patients (12.1%) had stable skin disease. Four patients (12.1%) patients had died by Day 28. No patient experienced progression of skin disease from baseline. Of the 25 patients without skin GvHD disease at baseline, 2 (8.0%) developed GvHD involving the skin at Day 28.

*Lower GI GvHD*

Overall, 31 (59.6%) of the 52 patients with lower GI GvHD at baseline (stage > 0) had improvement in their GI disease at Day 28, with complete resolution occurring in 15/52 (28.8%) patients; 12/52 (23.1%) patients had stable lower GI disease; 7/52 (13.5%) patients had died by Day 28; 2/52 (3.8%) patients with lower GI GvHD at baseline experienced progression of the GI disease. Of the 7 (14.3%) patients without lower GI GvHD at baseline, 1/7 (14.3%) had developed GvHD involving the lower GI at Day 28.

*Liver GvHD*

Overall, 11 (52.4%) of the 21 patients with liver GvHD at baseline (stage > 0) had improvement in their liver disease at Day 28, with 9 cases (42.9%) completely resolving; 5/21 (23.8%) patients had stable disease of the liver; 4/21 (19.0%) patients with liver involvement at baseline died by Day 28; 1/21 (4.8%) with liver GvHD at baseline experience progression of the liver disease. Of the 37 patients without liver GvHD at baseline, 3 (8.1%) had developed GvHD involving the liver at Day 28.

*Effect of continuing therapy*

Overall, 33/59 (55.9%) patients received > 8 Prochymal infusions and were included in the continuing therapy analysis. Of these, 18/33 (54.5%) were responders as they experienced additional benefit from continuing therapy. Of the 18 responders, 2 were complete responders who maintained their response through Day 100, and the other 16 showed additional improvement in GvHD (12 achieved a CR). When stratified by baseline GvHD grade 3/5 Grade B patients experienced additional benefit, 9/11 Grade C patients (81.8%) experienced additional benefit and 6/17 Grade D patients (35.3%) experienced additional benefit.

*Other endpoints*

*Overall response at Day 28 with respect to addition of 2nd agents:* OR at Day 28 in patients with no 2nd and with 2nd GvHD agents started within 1 week prior to study were similar (62.2% and 64.3%, respectively).

*Survival to 100 days in OR responders and non-responders at Day 28:* Of the patients with OR at Day 28, 75.7% (29/37) survived at least 100 days past the first infusion, while in those patients without an OR at Day 28 the corresponding survival rate was 31.8% (7/22). Survival through Day 100 as a function of Day 28 response was evaluated to determine the clinical value of the Day 28 OR endpoint. The patients achieving an OR at Day 28 had a 78% probability of surviving through Day 100, whereas patients who did not achieve an OR at Day 28 had 36% probability of survival.

*Survival through to 180 days following onset of GvHD:* Overall survival at 180 days following onset of GvHD was 62.1% (36/58). Kaplan-Meier survival analysis showed that the probability of survival at 180 days in the overall population (n = 58) was 65.2% (95% CI 52.9, 77.5).

*Comparison with historical control:* Overall, the patients treated in this study had a statistically significantly higher probability of survival compared with a historical control from the CIBMTR data base (p = 0.003).

*Emergency use patients:* Efficacy data for 12 paediatric patients treated under single patient, emergency use protocols are not useful for regulatory purposes.

### Safety

The focus is on safety data from the controlled clinical Study 280 because the placebo comparator allows control of confounding effects of underlying morbidities.

In Study 280, all patients experienced at least 1 TEAE in both groups.

The most frequently reported TEAEs occurring in ≥ 20% patients (Prochymal versus placebo) were peripheral oedema (35.6% versus 33.3%), abdominal pain (22.7% versus 17.23%), thrombocytopenia (22.1% versus 22.2%), hypokalemia (21.5% versus 13.6%), diarrhoea (22.9% versus 18.5%), hyperglycaemia (20.2% versus 13.6%), and pyrexia (20.2% versus 17.3%).

TEAEs occurring ≥ 5% more commonly in Prochymal group than in placebo patients were as shown in Table 9.

**Table 9: TEAEs occurring ≥ 5% more commonly in Prochymal group than in placebo patients (Study 280)**

| Study 280 | Prochymal | Placebo |
|---|---|---|
| Confusion & disorientation | 19.0% | 7.4% |
| Hypertension | 17.8% | 7.4% |
| Confusional state | 17.2% | 7.4% |
| Anorexia | 8.0% | 0% |
| Anxiety | 14.1% | 6.2% |
| Hypokalemia | 21.5% | 13.6% |
| Hyperkalemia | 12.3% | 4.9% |
| Abdominal distension | 9.8% | 2.5% |
| Staphylococcal bacteraemia | 12.9% | 6.2% |
| Tremor | 12.9% | 6.2% |
| Insomnia | 14.1% | 7.4% |
| Hyperglycaemia | 20.2% | 13.6% |
| Mucosal inflammation | 7.4% | 1.2% |
| Dyspnoea | 18.4% | 12.3% |
| Dizziness | 5.5% | 0% |
| Pneumonia | 12.9% | 7.4% |
| Haematochezia | 5.5% | 0% |
| Rash | 11.7% | 6.2% |
| Abdominal pain | 22.7 | 17.3% |

SAEs were reported in 89.5% Prochymal group compared to 87.7% placebo patients. The most commonly reported SAEs were as shown in Table 10.

**Table 10: SAEs in the Prochymal and placebo groups (Study 280)**

| Study 280 | Prochymal | Placebo |
|---|---|---|
| GvHD | 14.7% | 11.1% |
| Gastrointestinal haemorrhage | 7.4% | 6.2% |
| Sepsis | 8.6% | 7.4% |
| Intestinal GvHD | 6.7% | 3.7% |
| Pneumonia | 6.1% | 3.7% |
| Respiratory failure | 6.1% | 6.2% |
| Bacteraemia | 5.5% | 3.7% |
| Multi-organ failure | 2.5% | 7.4% |
| Pyrexia | 3.7% | 3.7% |
| Adenovirus infection | 3.1% | 1.2% |
| Acute myeloid leukaemia | 3.1% | 2.5% |
| Renal failure | 3.1% | 2.5% |
| Atrial fibrillation | 2.5% | 1.2% |
| Septic shock | 2.5% | 2.5% |
| Cytomegalovirus infection | 2.5% | 2.5% |
| Staphylococcal bacteraemia | 2.5% | 1.2% |
| Klebsiella bacteraemia | 2.5% | 1.2% |
| Renal failure acute | 2.5% | 2.5% |
| Respiratory distress | 2.5% | 1.2% |

The incidence of death was higher in the Prochymal group (64.4%) compared to placebo (54.3%). The proportion of deaths attributed to 'Infections & Infestation' was higher in Prochymal treated group (25.8%) compared to placebo treated patients (14.8%). The proportion of deaths attributed to 'immune system disorders' was also higher in Prochymal treated patients (14.1%) compared to placebo treated patient (6.2%).

No TEAEs related to ectopic tissue formation was reported in either treatment groups. TEAEs indicative of relapse of underlying disease were reported in 8% Prochymal and 9.9% placebo patients. There were no marked differences between the treatment groups as regards clinical laboratory tests, vital signs, or ECG results.

The TEAEs of 'psychiatric disorders' was reported in 54.6% Prochymal patients compared with 37% placebo group and underlies the higher incidence of AEs such as confusional state, anxiety and insomnia. All 'psychiatric disorders' SAEs were reported in 3.1% of Prochymal patients compared with 2.5% of placebo patients.

Two unexpected TEAEs occurring with higher incidences in the Prochymal group compared with the placebo group were hypertension and hyperglycaemia. Note however that at baseline, diabetes mellitus was notably more common in the Prochymal group (13.3%) than in the placebo group (5.7%).

### Risk management plan

See the accompanying TGA evaluation report.

### Sponsor's response

The sponsor has modified the proposed indication to restrict it to paediatric population as well as sought to reflect organ specific results (visceral or skin) in the indication. The proposal is essentially based on insistence that the results of the uncontrolled Study 275 be considered as confirmatory evidence of efficacy in place of controlled data in the Study 280. In doing so the sponsor argues that a more severely ill, refractory population was studied in the uncontrolled Study 275 compared to the randomised control Study 280 as shown in Table 11.

**Table 11: Comparison of severity of Study 275 and Study 280 populations**

| | Protocol 280 | Protocol 275 |
|---|---|---|
| Median duration of acute GvHD prior to start of study | 13 days | 30 days |
| Number of Failed Lines of Therapies for GvHD Treatment | 1 | median 3 |
| Grade D | 24% | 59% |
| Multi-organ involvement | 52.3% | 62.7% |
| Overall | moderate to severe GvHD | severe to end-stage GvHD |

The population treated in uncontrolled Study 275 had refractory GvHD despite prior treatment with multiple immunosuppressive agents. The objective in this study was salvage treatment in patients were non responsive to multiple lines of therapy. The expected outcome for these patients was death. According to this reasoning, achieving response in this population was unexpected and can be considered as true treatment effect despite its uncontrolled setting.

## Risk-benefit analysis

### Delegate considerations

This was an orphan designated drug. The estimate of incidence of aGvHD in Australia provided by the sponsor is as shown in Table 12.

**Table 12: Annual incidence of acute GvHD in Australia**

| | Adult + Pediatric | Pediatric (< 18 yrs) |
|---|---|---|
| Allogeneic HCT | **600-800** | **200-250** |
| **aGvHD Requiring systemic treatment (Grades B-D)** | 300-400 | 100-125 |
| **Refractory aGvHD** | 150-200 | 30-50 |

In Australia, there are currently no treatments approved specifically for aGvHD, although corticosteroids are considered standard of care for first line treatment.

The hMSCs can generally be transplanted without donor recipient HLA matching due to their immune privileged marker profile. The submission included *in vitro* data to support this contention.

The mechanism of action of *ex vivo* expanded hMSCs is thought to be due to the immunomodulatory, tissue protective and trophic effects of these cells. Various cell signalling and migration events associated with viable cells are proposed to contribute to therapeutic benefit. The *in vivo* proof of concept was limited based on the information included in the dossier.

The overall data package and the demonstration of net efficacy/safety benefit for the use of Prochymal in the proposed indication including restricted use in paediatric population does not appear to be adequate for the reasons noted below and for which advice from the ACPM is requested.

### *Quality and manufacturing data*

Although outstanding quality control issues were resolved in light of the PSC recommendations and the quality evaluators have no final objection to the registration of Prochymal, the recommendations include a number of areas for which the final plan/tasks to be undertaken by the sponsor have not been submitted or reviewed. A notable case is consistency of certain testing of the DS and for the finished product. Similarly, a number of manufacturing process changes were implemented in 2009 and were thus subsequent to the completion of the process validations for the then finished product. After concern were raised by the TGA evaluators and endorsed by the PSC, the sponsor has agreed to reinstate a number of process steps. The quality evaluators advise that reinstatement of these steps is sufficient to control the current manufacturing process. However, they also inform that:

**changes have been introduced to the manufacturing process for Prochymal in response to issues raised by TGA. On this basis, the manufacturing process utilised for generation of currently held stock of Prochymal is not in compliance with TGA's requirements and should not be released in Australia.**

This implies that the finished product used in the clinical trials will not be consistent with the finished product proposed for registration and release in Australia. It is well known that subtle changes in manufacturing can significantly alter efficacy and safety characteristics of a ('conventional') biological product such as a vaccine or a monoclonal antibody. How this principle applies to cell based products is unclear. However, there is a concern with regard to the validity of the clinical data generated using the earlier formulation. It is also indicative of the fact that Phase 3 clinical data generation was undertaken without first fully finalising the final formulation.

### *Nonclinical data*

The nonclinical data package does not support approval mainly due to inadequate demonstration of mechanism of action, lack of demonstration of preferential homing, and failure to adequately count and characterise naive or differentiated cells in target (affected) tissues as well as non target tissues. The purported mechanism of action necessitates living cells for their biological presence but this has not been adequately proved and may have been refuted.[66]

Other issues not addressed in the nonclinical package involved deficiencies such as number of cells used, capability of cell population to secrete factors of relevance, that is, identity, quantification, concentration, and diffusion potential of secreted factors of relevance.

Overall, the nonclinical data included *in the dossier* do not allow reliable interpretation and extrapolation with respect to the proposed mechanism of action, the proposed dose and its clinical use.

### *Clinical data*

In a pragmatic approach, while issues relating to basic science are clarified overtime by ongoing research, the aforementioned deficiencies in the nonclinical data package would not hinder approval if there was unequivocal proof of efficacy in the clinical testing programme. However, the clinical efficacy/safety could not be clearly demonstrated based on the following findings.

*Clinical efficacy (controlled data)*

The pivotal efficacy Study 280, which was a randomised placebo controlled trial, failed to demonstrate a statistically significant difference between Prochymal and placebo for the

[66] Tse WT, et al. (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75: 389-397.

primary efficacy endpoint of CR of ≥ 28 days duration (all aGvHD grades), that is, 34.7% (60/173) versus 29.9% (26/87), respectively (Treatment Difference 4.8%; 95% CI -7.3% to 16.8%; p = 0.42). The OR (CR + PR) (all aGvHD grades) was 57.7% (94/163) versus 50.6% (41/81) at Day 28 (p = 0.22) for the two groups, respectively. There was also no survival (> 100 days) benefit (all aGvHD grades), that is, 52.1% (85/163) versus 50.6% (41/81) for the two groups respectively (p = 0.78) or survival (> 180 days) benefit (all aGvHD grades), that is, 34.4% (56/163) versus 42.0% (34/81) for the two groups, respectively (p = 0.27).

*Clinical safety*

The comparative adverse reactions profile appeared to be worse for Prochymal treated patients compared to the placebo treated patients including mortality in Study 280.

*Uncontrolled data*

The uncontrolled data in the paediatric Study 275 does not allow reliable assignment of causality, not withstanding comparison with a historical control that was not concurrent. The Delegate does not accept the argument that any response in this population represents true treatment effect due to the severer form of disease. In the Delegate's view, this represents a speculative expectation of efficacy and a more reliable guide to the true treatment effect in paediatric patients is the subgroup of 28 children treated in the Study 280 which suggests age effect (lower response in adults than in children) rather than differential effect related to the severity of disease. As apparent from showing multiple efficacy outcomes in this paediatric subgroup (Table 7), a range of clinically meaningful treatment effect sizes from 14% to 28% above an otherwise high placebo response. The issue here is clearly a lack of power due to small numbers, although a baseline imbalance in the age profile (6 years in Prochymal group compared to 9 years in placebo group) may have caused some confounding in favour of Prochymal. It will be useful to have these data included in a future pooled (preferably prospectively designated) analysis if more controlled data become available. It will also be of interest to compare the 64% versus 43% CR of at least 28 days duration in Prochymal and placebo paediatric subgroups, respectively, in Study 280 with CR for at least 28 days in Study 275, which was not reported. It is also not clear why recruitment in Study 280 was stopped in favour of uncontrolled treatment in Study 275. This was an unfortunate as the failure to reach statistical significance due to low study power shows despite clinically relevant large effect sizes in the paediatric subpopulation.

Advice from the ACPM is requested.

## Proposed action

Given the above findings and my comments, the Delegate is of the view that the current submission has demonstrable deficiencies in all areas – that is, Quality, Toxicology and Clinical – and thus its efficacy and safety were not satisfactorily shown based on the data in the submitted dossier. The Delegate accepts the drug will be used in highly specialised units by highly trained physicians. However, this in itself is not an alternative to reliable evidence of effectiveness for general marketing authorisation.

Please also note that remestemcel-L (*ex vivo* hMSCs), as product derived from blood, is an unscheduled substance under the Poisons Schedule, that is, it has not been classified as prescription only medicine. However, given the expected use of this drug only in specialised circumstances this is not considered an issue.

Please also note that such submissions now fall under the new Biologicals regulatory framework whereby an orphan designation is no longer available which may be relevant to any future submission for this product.

The Delegate thanks the Committee for considering this submission and providing advice to the TGA.

### Outcome

On 18 November 2012, Delpharm Consultants Pty Limited wrote to the TGA, asking for the application for Prochymal to be withdrawn before it was presented for consideration by the Advisory Committee on Prescription Medicines.

# Attachment 1. Extract from the Clinical Evaluation Report

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