

Cell Processing for Clinical Trials and Commercial Manufacture

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Cellular therapies (CT), cell-based therapies, tissue-based therapies, and tissue engineering encompass a broad, rapidly growing field of medicine that involves the manipulation and administration of cells for the treatment of disease. This field can be categorized in various ways, based on 1) the starting cell population (e.g., embryonic stem cells, adult stem cells, islet cells), 2) the type of cells generated in the process (e.g., dendritic cells, chondrocytes), 3) the disease or organ targeted (e.g., cardiac disease, diabetes), 4) the type of manipulation (e.g., cell expansion, gene therapy) or, 5) the complexity of manipulation (i.e., basic to highly sophisticated) (Table 1).

Common features of all CT are requirements that source cells be identified, collected, processed, stored, transported, and administered. Each step must incorporate

procedures that ensure that the integrity of the end cellular product is maintained. Thus, one should approach the production of a CT no differently from the production of a pharmaceutical drug or medical device — including requirements that the entire process occur in appropriate physical environments with protocols and procedures linked to high-level quality systems.¹

The manipulation of human cells to produce cellular therapeutic products is, with a few exceptions, still considered an experimental therapy. Thus involvement in clinical trials exploring various aspects of CT is appealing to any academic centre involved in clinical research. In 1996, the Peter MacCallum Cancer Centre established the Centre for Blood Cell Therapies (CBCT) to support a range of activities involving cell and tissue manipulation under conditions that would meet the requirements of the code of current Good Manufacturing Practice (cGMP). We describe the principles and practicalities of developing a cGMP-compliant cell processing/engineering center (CPC) capable of manipulating and processing human cells for clinical trials and related commercial activities.

Scope of Activities

It is an extraordinarily difficult task to predict the future of CT. Although cellular products such as autologous cultured chondrocytes and cultured epidermal autografts are approved for commercial use, the recent events surrounding the controversies of gene therapy highlight the unpredictable nature of the CT field.²⁻⁸ We elected to design and construct a facility that would remain as “generic” as possible, to enable adaptation to potential future demands of CT. This design meets the technical and regulatory demands required to sustain cells in short- and long-term culture (process cleanroom) as well as meeting the sometimes conflicting requirements for gene transduction protocols (process cleanroom, containment). Because of our own long-term research objectives, we also incorporated more specialized equipment, such as a high-speed cell sorter for rare target cell isolation, within the cleanroom environment.

We anticipated that regulatory authorities would require that cGMP standards (or close equivalents) be applied across the whole spectrum of the cell manipulation process, including acquisition of the starting cell population (e.g., apheresis), cellular isolation, processing, and

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Table 1. Clinical Applications of Cell Therapies¹

Disease States	Cell Therapies	
Cancer		
	Hematopoietic Stem Cell (HSC) Transplantation	
	Autologous and allogeneic HSC	
	Ex vivo expansion of HSC	
	'Suicide' T cells – gene transfer	
	Mesenchymal stem cell transplantation	
	Immunotherapy	
	Dendritic cells	
	Macrophage-activated killer cells	
	NK cells	
	NK/T cells	
	T cell expansion	
	Co-stimulatory molecules (gene transfer)	
Orthopedic		
	Expanded chondrocytes	
	Mesenchymal stem cells	
Neurodegenerative disorders/trauma		
	Adult stem cell-derived neural cells	
	Embryonic stem cell-derived neural cells	
Cardiovascular disease		
	Infusion of marrow/blood-derived angioblasts	
Organ replacement		
	Pancreas (diabetes)	
	Pancreatic islet cells	
	Embryonic stem cell-derived islet cells	
	Adult stem cell-derived islet cells	
	Liver (failure, metabolic disorders)	
	Bioartificial liver	
	Isolated hepatocytes	
	Hepatocyte stem cells	
	Kidney (failure)	
	Bioartificial kidney	
Wound healing		
	Keratinocytes	
	Skin stem cells	
Infectious diseases		
	Antigen-loaded dendritic cells	
	Lymphocyte expansion	
	Macrophages	
Genetic deficiencies		
	Hemophilia	Gene therapy
	SCID	Gene therapy
	Cystic Fibrosis	Gene therapy
Autoimmune diseases		
	Immunotherapy	
	Dendritic cells	
	Lymphocyte expansion	
	Natural Killer cells	

HSC, hematopoietic stem cells; NK, Natural Killer; SCID, severe combined immunodeficiency

storage. Thus, we formed an umbrella organization — the Centre for Blood Cell Therapies (CBCT) — that would possess a GMP license covering all the relevant aspects of cell processing. The quality system (QS) was designed with platform generic standard operating procedures (SOP) that could be modified or added to for specific processes to be performed within the CPC (Fig. 1).

Regulatory Licenses

In Australia, the regulatory requirements for CT are similar to those in the United States, Canada, and Europe. The Australian Therapeutic Goods Administration (TGA, Australia's equivalent to the US FDA) is the government regulatory body that determines whether a facility complies with cGMP requirements.⁹⁻¹³ The TGA is a unit of the Federal Department of Health and Ageing and is responsible for administering the provisions of the Australian legislation for blood and related bio-materials. The TGA carries out a range of assessment and monitoring activities to ensure that therapeutic goods available in Australia are of an acceptable standard. The TGA website (www.tga.gov.au) details the background and current regulatory environment, which in the case of CT is in a rapidly-evolving mode.

The CBCT has worked collaboratively with the TGA and other regulators (including the Office of the Gene Technology Regulator and the Gene Therapy Research Advisory Panel of the National Health and Medical Research Council) to try to ensure that the CBCT meets current and future regulatory requirements. Ensuring ongoing compliance with the evolving standards for cell- and tissue-based therapies required specific decisions during facility design and in the selection of the QS standards employed. The CBCT was the first cell processing facility in Australia to obtain TGA approval for its operations.

Quality Systems Provider and Systems

We have established a formal long-term collaboration (including independent arrangements for quality assurance monitoring and audit) with the Australian Red Cross Blood Service (ARCBS). This involves the joint appointments of a quality systems manager (QSM) and a quality systems officer (QSO) to manage the CBCT QS along with a document control function provided through ARCBS (Fig. 1). We have a comprehensive QS for 'routine' products, such as peripheral blood progenitor cell collection and processing, through to novel experimental products produced for clinical trials. To date, in peer comparisons, we have found that our QS have met or exceeded those of our local and international collaborators.

An extensive documentation system for relevant protocols and procedures within the CBCT has been developed to comply with cGMP requirements. In addition to covering all stages of production, namely collection, processing, storage and release, it also addresses the fol-

lowing elements: equipment (purchase, use, and maintenance); materials (specifications, quarantine, and release); and quality systems (qual-

ity control, validation, and document control). Where our system interacts with hospital infrastructure departments, such as engineering

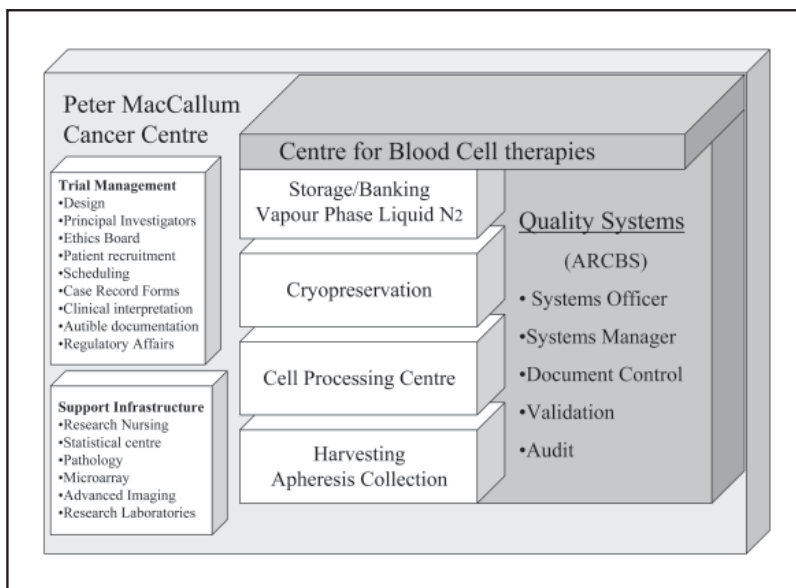


Figure 1. **Model of the Centre for Blood Cell Therapies (CBCT) within an academic centre – the Peter MacCallum Cancer Centre.** The CBCT is licensed in all aspects of cell procurement, processing, and storage. The quality systems are overseen through collaboration with the Australian Red Cross Blood Service (ARCBS). The CBCT links closely with trial management facilities and the hospital's support infrastructure.

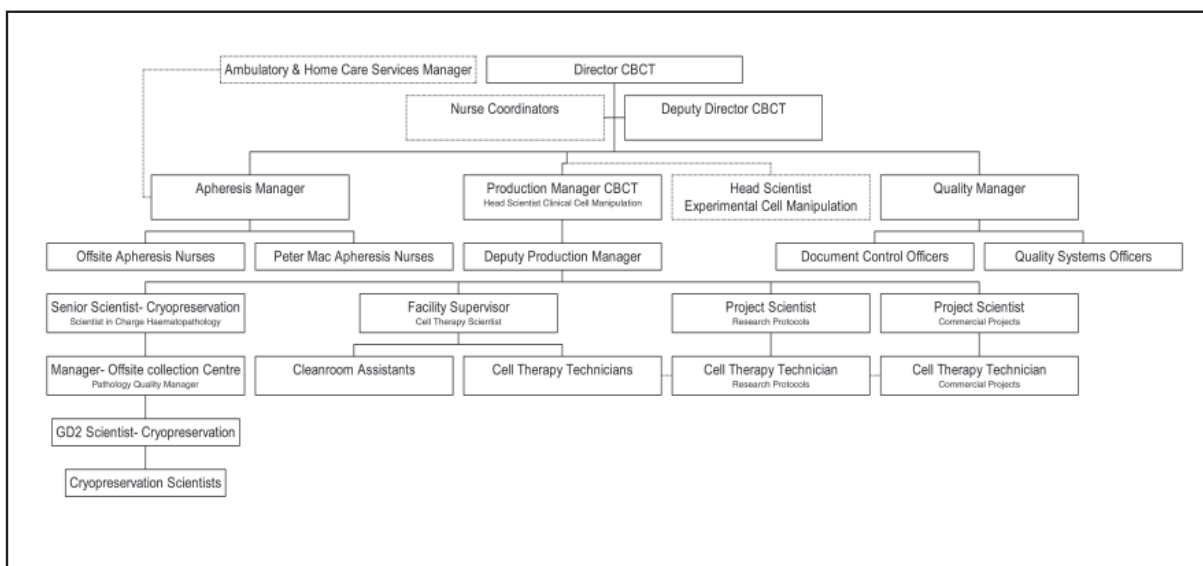


Figure 2. **Organizational Chart of the Centre for Blood Cell Therapies.** The structure meets the requirements of GMP regulation and hospital organizational structure. The two arms consist of 'production' (through the production and apheresis managers) and 'quality systems' (through the Australian Red Cross Blood Service).

and purchasing, service level agreements (SLA) have been established. We have a formal training and continuing education and competency assessment system for staff, which includes learning modules, training and assessment, and procedural change management. Technical Master Files (TMF) have been produced for autologous blood stem cells, mononuclear cells, dendritic cells, and gene transfer protocols.

Staffing

The CBCT staffing is detailed in the organizational chart (Fig. 2), which conforms to cGMP requirements. Specifically, there is a production arm that includes CBCT staff in apheresis, cryopreservation, and the cell manipulation laboratories. To maximize flexibility, we staff the cell manipulation laboratories with hospital pharmacy technicians who have additional cell manipulation

training and are employed on an ‘as needed’ basis. The second arm of the organization, QS, is staffed by ARCBS.

Clinical Trials in the Academic Environment

Our approach to CT trials builds on the infrastructure we have established for pharmaceutical drug trials. This includes capacity to submit trial protocols, obtain review by an institutional bioethics committee, recruit sufficient patients, and track and document outcomes that meet the requirements of good research practice. We seek to be commercially competitive with the aim of providing clinical trial outcomes in a cost-effective manner. Moreover, we recognize that it is critical that the CPC remains close to the clinical interface, strongly linked to the source of patient referrals. Consequently, we developed a CPC within our

academic centre that is committed to clinical research, free from commercial ties, and committed to its own QS infrastructure. By having a CPC within our centre, we can provide ongoing physician input into the CT trial design, ongoing contact with regional clinician colleagues, and closer contact to the patient, hence a higher likelihood of achieving trial recruitment targets. Our academic institute brings substantial expertise in fundamental research to any clinical trial, and therefore the potential to add value to research collaborations. Examples include involvement of the Stem Cell Research and Cancer Immunology Laboratories and the advanced imaging department. The latter has been involved in studies tracking the fate of infused cells (see “*In vivo tracking...*” section). Moreover, we can share our intimate knowledge of local, regional, and international regulatory requirements for CT; a valuable resource for companies seeking to commercialize their product. The value of this consultative role cannot be overemphasized, particularly for smaller companies entering the field of CT or those targeting an international market for their product.

Commercialization

All CT studies are by their nature expensive and labor-intensive. Furthermore, in a rapidly-changing field we must respond quickly to the needs of our collaborators. To engage in new CT initiatives we need to quickly and accurately determine costs and be able to commit resources in a timely fashion. Thus, we formed a separate business structure, Cell Therapies Pty Ltd., to facilitate commercialization of the CBCT. This special-purpose, independent company, with majority ownership by the Peter MacCallum Cancer Centre, has been set up to manage the commercial interface to the CPC. Cell Therapies Pty Ltd. provides clients and collaborators with a focused business unit that

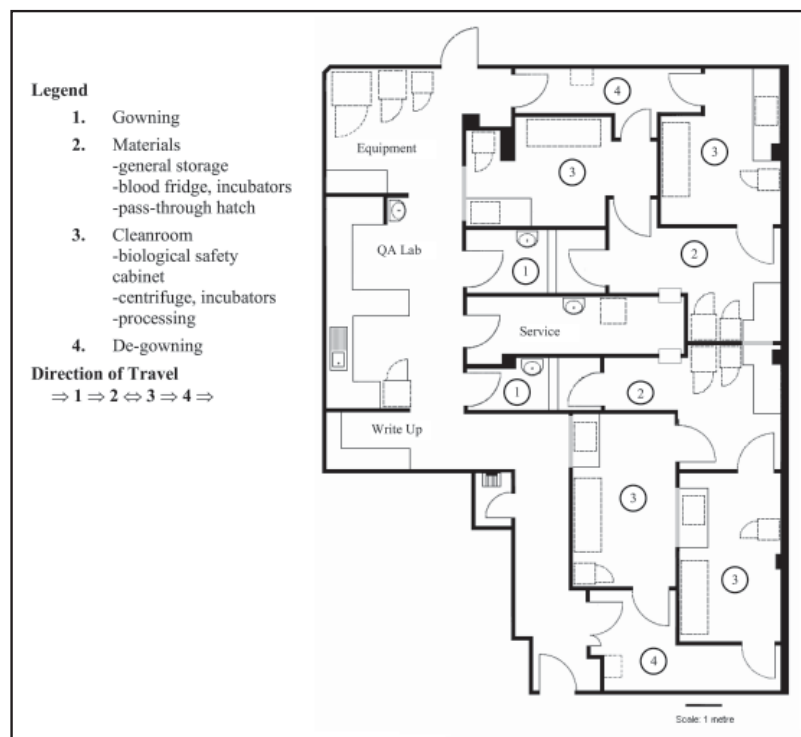


Figure 3. **Cell Processing Centre (CPC) design.** This includes the four generic cell processing suites (within each are gowning, materials, cleanroom, and degowning areas), QA, write-up, and some of the storage areas.

is able to negotiate and execute all business aspects of contract engagements. We are able to provide access to the hospital's wider expertise in advanced imaging, clinical trial design and approval, recruitment of principal investigators and patients, licensed manufacture, audit compliance, clinical interpretation, and reporting. We build on our experience in protocol development and SOP preparation, while matching research imperatives to licensed production restraints.

The Cell Therapies Facility — Laboratory Design

The CPC is the most tangible aspect of a CT facility and it must conform to a variety of requirements to meet the demands of GMP. Such requirements have been extensively reviewed elsewhere.¹⁴

As shown in Figure 3, the total space of these laboratories occupies approximately 218 m². Production is undertaken in four Class 10,000 (ISO 5, Class 350) fully-equipped cleanrooms containing Class 100 (ISO 7, Class 3.5) workstations. These rooms average 12.5 m² each and are designed to facilitate flexible, protocol-specific configurations. The rooms can operate independently under positive or negative pressure and the facility meets all physical containment level 3 (PC3) international standards.

There are also two separate cell sorting laboratories — a 23-m² high-speed cell-sorting laboratory suitable for research activities equipped with FACStar Plus, FACS DiVa, and FACScalibur, as well as a second high-speed cell-sorting cleanroom, 20 m², set up for clinical cell sorting for patient use, equipped with FACS Vantage SE (Figs. 4 & 5).

In addition to the cleanrooms, there are change rooms, gowning and de-gowning areas, and conventional laboratory space such as writing-up areas, quality assurance testing areas, and a refrigeration and transfer zone.

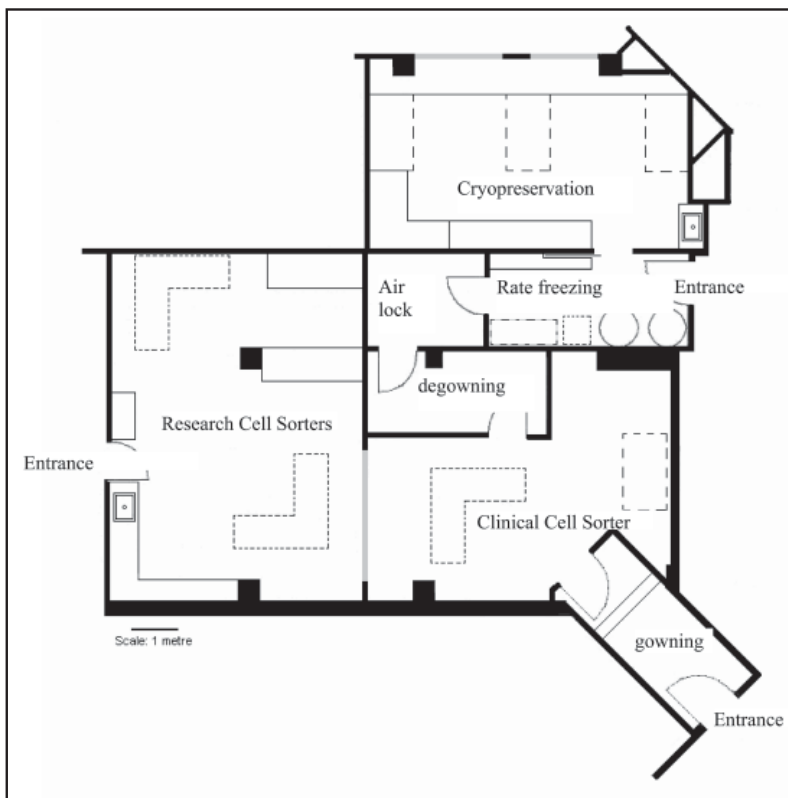


Figure 4. **Clinical and Research Cell Sorting and Cryopreservation Laboratories.** Separate entrances for staff into each of the areas.

The facility is fully computer controlled and monitored 24/7 to comply with and exceed regulatory requirements for day-to-day operation and audit traceability.

In addition to (and separate from) the cleanroom facilities is a cryopreservation facility equipped with three workspaces suitable for simultaneous processing of bio-materials and two rate-controlled freezers. For long-term banking, there is a vapour-phase liquid nitrogen storage facility equipped with five auto-fill 340-liter tanks supplied via insulated vacuum lines from a 3,800-liter supply tank (not shown).

The TGA has specified that apheresis is to be an integral part of the regulated manufacturing environment. The apheresis unit falls within the direct control of the CBCT with a fully-equipped four-room apheresis suite.



Figure 5. **Cell Sorting.** Patient cells are sorted using the FACS Vantage SE within the cleanroom.

Special Laboratory Features

Air containment. Air arrives into each room through ceiling-mounted, high-efficiency particulate air

(HEPA) filters while all departing air leaves each room via low-level HEPA filters. The rooms have been tested with air pressure differentials of over 100 pascals to ensure they are leak-free at every point. Doors are sealed when closed to further prevent air leakage.

Double barriers. Further safety is assured through the use of biological safety cabinets within these cleanrooms, which further filter incoming and exiting air with their own HEPA filters.

Individualised room pressure control. Each air supply and departure point has a variable-speed fan controlling the amount of air coming in and out of each room. These fans are computer controlled through a building automation service that also records the pressure within each room. This allows a pressure to be set for that room and for that pressure to be maintained — even as doors are opened.

Positive and negative pressure control. Further safety is built in with the use of pressure gradients.

When carrying out procedures that require high degrees of containment, the pressures can be sequenced so that there is a large negative pressure within the cleanroom, ensuring that all airflow is toward the cleanroom. The facility can operate in both modes simultaneously, with two cleanrooms operating in positive mode and the other two operating in negative pressure mode.

Access control. The facilities are located in an area that is not accessible to patients or visitors. Entry to the main laboratory zone requires an appropriately coded card, while access to each pair of cleanrooms is further restricted by a digital combination lock. Entry and exit doors are electronically interlocked to prevent both doors and either entry or exit anterooms from being simultaneously opened. One-way doors are used in the anterooms to prevent backtracking from ‘dirty’ to clean areas.

Equipment and environmental control. Should pressure control fail in any room, facility alarms will sound. Computer displays are

present in both parts of the facility showing the exact pressure in each room and the status of all plant equipment.

Clinical Trial Portfolio

As described, an original aim of the CPC was to be involved in a variety of clinical trials. Like pharmaceutical drug trials, these take the form of Phase I through Phase IV studies (Table 2). One important difference for CT clinical trials is the ability to perform trials based on in-house research, and to enter into partnerships for commercial production of approved cell therapeutics. Current trials based on translating in-house research include *ex vivo* expansion of hemopoietic stem cells (Fig. 6) and expansion of autologous T cells. Collaborative trials funded by peer-reviewed grants include dendritic cell (DC) therapy for adenocarcinomas and hepatitis C, Parkinson’s disease, and ischemic heart disease. Commercial-sponsored trials include Phase III studies of monocyte-activated killer cells (MAK) including tracking studies with these cells and DC trials in melanoma and myeloma (including tracking studies). Production of chondrocytes and an IL-2 agonist as a commercial therapeutic for osteoarthritis are also underway.

In vivo Tracking Studies Utilising Labeled Cells

One advantage of a CPC within an academic centre is the access to other research tools that add value to a clinical trial. One particularly striking example is the capability of our fully-integrated, multi-modality, advanced imaging facility to identify and track the fate of infused cells at high resolution. This provides uniquely valuable data for patient monitoring and for evaluating product potency *in vivo*.

We have performed multiple tracking studies of a variety of

Table 2. Clinical projects within the Centre for Blood Cell Therapies

Cell Therapy	Disease States	Type of Study	Comments
Autologous HSC	Hematological cancers	Phase II-IV	
Autologous HSC	Cardiac failure	Phase I	
Ex vivo expansion of HSC	Breast Cancer	Phase II	
Dendritic cells	Melanoma	Phase III	
Dendritic cells	Myeloma	Phase II	Cell tracking
Dendritic cells	MUC-1 positive tumors	Phase II	
Dendritic cells	Hepatitis C	Phase I	
MAK cells	Ovarian Cancer	Phase III	
MAK cells	Ovarian Cancer	Phase II	Cell tracking
Mesenchymal stem cells	Bone grafting	Phase I	
Neural stem cells	Parkinson’s disease	Phase I	
IL-2 agonist	Osteoarthritis	Commercial	
Chondrocytes	Osteoarthritis	Commercial	

HSC, hematopoietic stem cells; MAK, Monocyte-derived activated killer cells



Figure 6. **Ex vivo expansion of hematopoietic stem cells.** Static cultures of stem cells grown for 12 days in combination with cytokines and expansion media within cleanrooms.

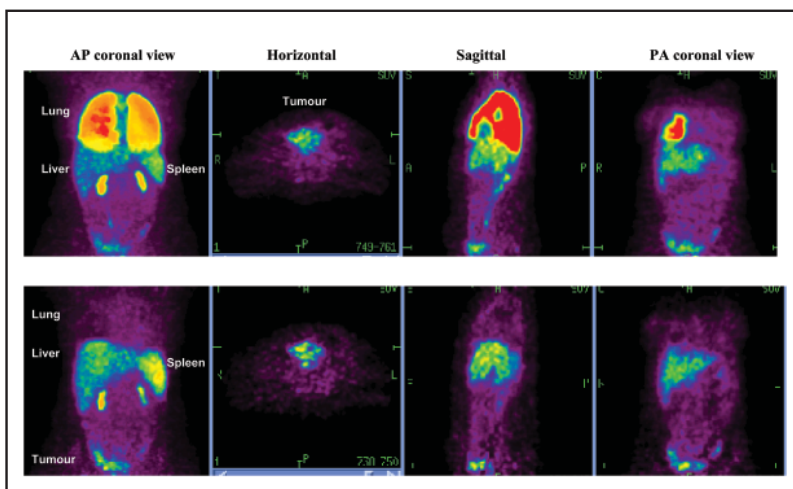


Figure 7. **Cell tracking.** Patient's modified effector cells have been labelled with F^{18} -fluorodeoxyglucose (FDG), infused intravenously, and scanned by positron emission tomography at 1 hour (upper panel) and 4 hours (lower panel). Following lung clearance, cells are seen to track to liver, spleen, and to the known site of tumor in the pelvis.

cells using custom cell labeling and an array of imaging technologies, including CT, positron emission tomography (PET), and single photon emission computed tomography (SPECT) (Fig. 7). Combinations of tomographic and 2D whole-body imaging can deliver spatial infor-

mation at previously unattainable resolutions, and can monitor cells *in vivo* over hours to days.

Future Directions

To enhance recruitment to clinical trials we are currently extending

our license to incorporate remote site apheresis collection and transfusion at other hospitals. We also are in the process of developing a Commonwealth Government-funded Australian Network for cell therapies involving three other medical centres in capital cities on the eastern seaboard of Australia.

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