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PROSPECTS

Concept and Organization of a Clinical Gene Therapy Lab

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Abstract Designing a dedicated clinical facility to meet the needs of existing and developing Gene Therapy Protocols presents a unique challenge. Here, we review some of the issues we faced and share some of our design concepts. An optimal Clinical Gene Therapy Lab must meet relevant regulatory guidelines, interface with other hospital labs as well as the clinic and patient care areas, be efficient and flexible in utilization of space, and have the potential to meet future needs without continual renovation. As clinical science expands to include more gene transfer approaches, specific laboratory areas for this type of work will become increasingly necessary. © 1995 Wiley-Liss, Inc.

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Performing gene therapy protocol manipulations in a clinical setting presents some unique problems. The clinical gene therapy lab must have the rigor and standardization of a hospital blood bank, the flexibility and equipment of both a molecular genetics laboratory and tissue culture facility, and the attention to detail and quality standards of a pharmaceutical production facility. Most producers of vectors and other reagents used in gene therapy protocols have established dedicated manufacturing and testing facilities. However, once the materials are received at the institution performing the trials, a dedicated on-site area is required. Performing this type of protocol is often not appropriate in a clinical lab used for blood banking or cryopreservation. Although most clinical facilities will not be performing large scale production of vectors (usually in the form of viral supernatants) because of the impractical aspects of testing and quality control, a "pharmaceutical" is, however, being manufactured—human cells are removed, altered, and then returned to patients. While one option is to convert regions of existing clinical lab space in order to attempt to meet these needs, we have chosen to design a facility specifically for the purposes of performing human gene therapy protocols. This facility will meet current needs and provide the flexibility to develop new protocols.

Gene therapy is not a isolated activity. It is part of a concerted, "bench-to-bedside" approach to the most advanced patient care. There are other aspects to this as well, and this is the rationale behind the approach taken by the Cancer Center team at UMMC. We have created a "LINK" between lab and clinical research, and the results is four LINK labs which are of a dual clinical-research nature and an integral part of the new UMMC Cancer Center. The UMMC Cancer Center LINK Lab system is comprised of four laboratories which are located in a suite with shared maintenance facilities, like a glass washing suite, and good access to the bone marrow transplant unit and the research labs. The LINKs are: 1) Cryopreservation and Stem Cell, for cell processing/purging, storage of bone marrows, stem cell assays, and cell banking; 2) Gene Therapy, for culture of human tissues, viral transductions, analysis of samples for presence of vectors, storage of transduced cells, and development of new vectors and systems; 3) Cancer Molecular Genetics, for banking of RNA and DNA from tumors, analysis of gene expression in malignant tissues, and detection of transforming agents/viruses; and 4) Cytokine and Cytokine Receptor, for analysis of cytokine and cytokine receptor expression in normal and malignant tissues. Each lab is under the direc-

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tion of a faculty member who is affiliated with the Cancer Center and also pursues an independent but related area of basic science research. These labs provide a bridge between basic science research and clinical application and an available research base for all members of the Cancer Center, whether basic scientist or clinician—hence the name LINK Labs. This type of multidisciplinary scientific investigation provides the type of environment which maximizes resources and yields the most innovative research—just exactly the type of setting needed for gene therapy research.

The points to be considered when designing a lab to be used for clinical gene therapy are essentially the same as those for designing a clinical or applied research laboratory. They are 1) functional adjacencies (locations of equipment and labs), 2) path of transfer (where will material begin and end up), 3) flexibility and convenience (future needs, all protocol features), and 4) safety issues (and regulatory guidelines).

FUNCTIONAL ADJACENCIES

The location of an entire laboratory is as important as the placement of the equipment inside the laboratory. In the case of the Gene Therapy Lab, location issues include proximity to the BMTU (bone marrow transplant unit) which will be the primary site of delivery for gene therapy products to patients. The intention is to reduce the transport distances of patient samples. Location near the other LINK labs is also important since they are also involved in some protocol aspects, and the staffs share weekend coverage for sample pickup and emergencies. Within the laboratory, issues include minimizing the traffic through areas where the patient cells are cultured and providing each work area with the required equipment in a functional arrangement.

Location of the laboratory is within a suite of Cancer Center labs (LINK labs). The entire suite is entered from a secondary hallway near a service elevator with staff-only access; the BMTU is on another floor but is very close to this same elevator, which reduces the actual linear distance for samples to travel. There is a small office which opens to the public hallway; this will be used for mailboxes and central services for the labs. Entry to this laboratory suite is obtained through a secured entrance; this is a recommended precaution for labs which process and store patient materials or handle biohazard reagents. Once inside the suite there is a central corridor, showers and lockers, small conference room, four labs and offices, and a glassware/autoclave suite which can be entered through the LINK lab corridor or through a one-way exit from the Gene Therapy Lab anteroom. The entire suite has its own air, plumbing, vacuum, and electrical systems.

Within the Gene Therapy Laboratory, foot traffic patterns have been established so that areas which are to be used for handling cells are isolated (see Fig. 1). A pair of tissue culture bays (see diagram), which are U-shaped and completely self-sufficient, are located at the back of the laboratory. Each bay contains LAF hoods, incubators, water baths, microscope, benchtop centrifuge, and other items needed to culture and maintain cells. If necessary, each could be further isolated by installation of a partition. The molecular genetics work area is located just inside the entrance and is positioned as a barrier to accessing the rear sections of the lab. Storage and common use equipment is located around the perimeter of the laboratory, where it can be reached by all users without interfering with other areas. All storage shelves and cabinets have glass doors which permit viewing of contents and are easily wiped down with disinfectants when the lab is serviced. Lab servicing is described by a detailed protocol which includes everything from floor cleaner types to wiping off every surface in the laboratory, including things sitting out on open shelves. Much of the equipment is covered by washable or removable plastic sleeves.

Many decisions about laboratory layout were based on safety issues and will be discussed in that section. Briefly, the product (human cells in this case) is isolated, not only in the tissue culture bay, but also with a one-patient-per-incubator system which extends to include hood use time and dedicated supplies. The PCR work station enclosure is located outside the room where the cells and vectors are cultured. By placing this station in an adjoining room, we hope to avoid the potential background problems that could be created by doing amplification of viral sequences in the same room in which they are grown.

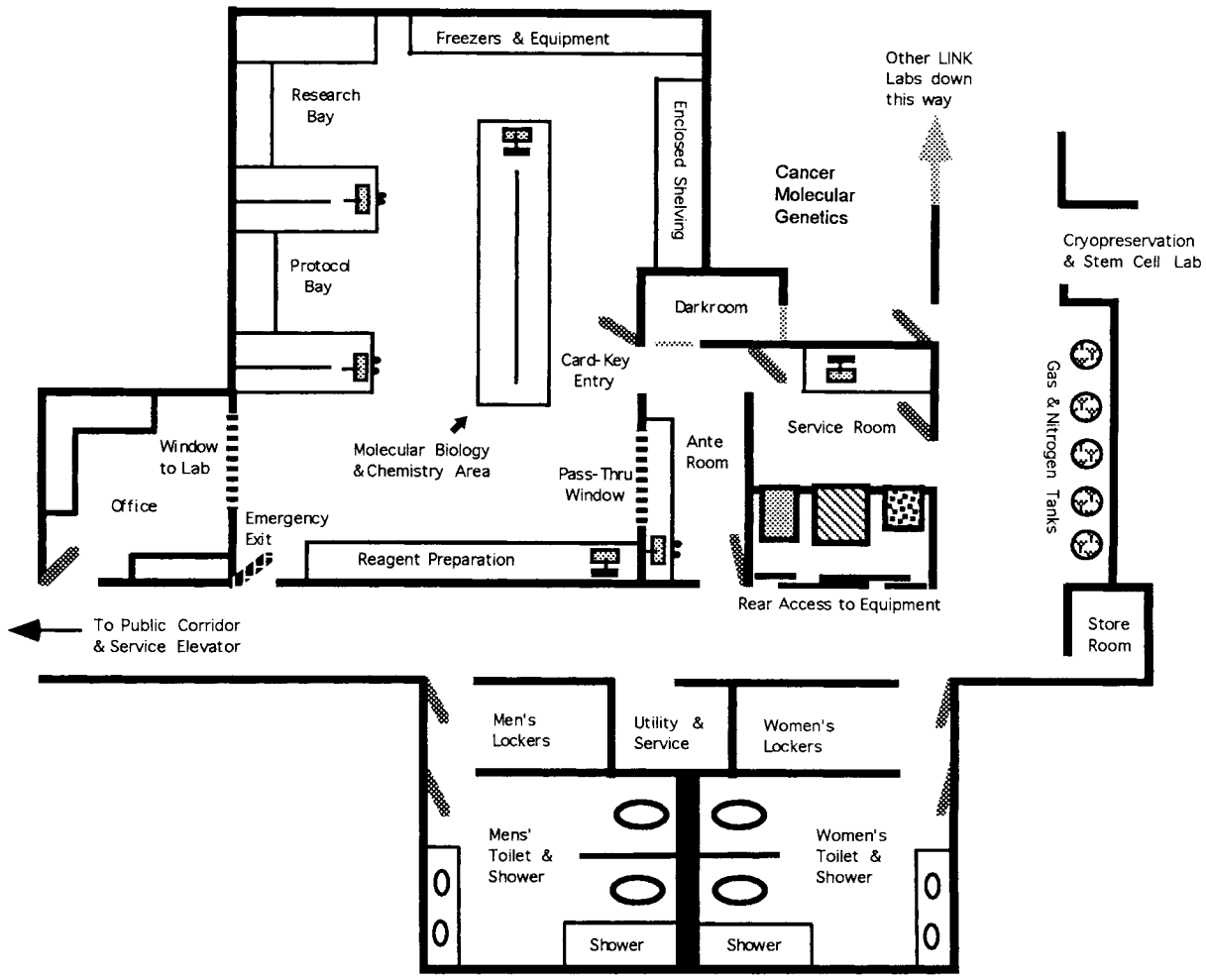

Fig. 1. A diagram of the Gene Therapy Lab layout. This is a general figure of the lab layout. The interior main Gene Therapy Lab is about 1,000 square feet of lab space, divided into two isolated tissue culture work bays which are located behind the molecular genetics work area. The office has a see-through window into the lab for convenience, and the lab has a pass-through window for waste and trash removal. The corridor and service areas are shared with the other LINK Labs in the suite, but installation of doors and partitions in the corridor can extend the controlled access lab space to include these as well.

PATH OF TRANSFER

The concept of path of transfer is based on the locations and manipulations of the material from the time it leaves the patient until it reaches its final destination. In this case the path is from the donor, to the lab, within the lab(s), to storage or analysis, into a patient, and then either remaining in the patient or being part of an analytical sampling removed from that patient (see Fig. 2). The final destinations in this case are analysis prepatient, remaining within patient, or being withdrawn from patient for analysis. All of these points, with the exception of those cells remaining within the patient, can be dealt with in considering lab design. The issue of altered cells which remain inside a patient, who then leaves the trial site to return to the community, is one best dealt with by the local and national regulatory officials who approve such trials.

Removal of material from a donor/patient is arranged in conjunction with the BMTU and the Cryopreservation and Stem Cell Lab, which requires coordination and communication between nurses and the staff in the labs. Scheduling and prioritizing of protocols is handled by weekly meetings of the LINK Labs directors and members of the Executive Clinical Research Committee of the Cancer Center. The route materials will travel and the type of containers

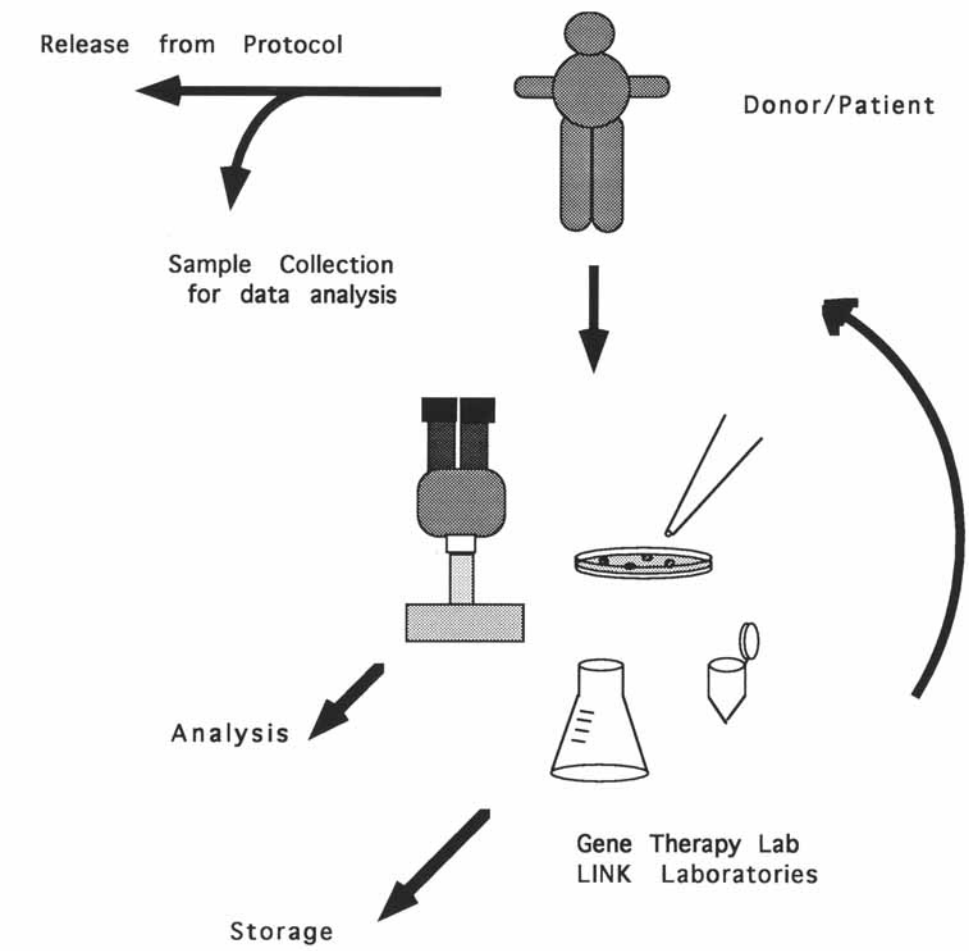

Fig. 2. Path of transfer. This illustrates some of the possible paths followed by material involved in the current gene therapy protocols. Each of these sites must be considered in both lab and protocol design.

must be cleared with hospital biosafety and infection control, as with any moving of organs or tissue. We have located the lab in such a way as to minimize actual travel distances and eliminate use of public elevators. All samples and collection devices (syringes, etc.) are carried inside nonbreakable plastic boxes with a silicone gasketed lid and metal closures. These boxes are leakproof, available in several sizes, and permanently marked with biohazard labels as well as lab locations and phone numbers.

Once inside the lab suite, material which is destined first for processing or preservation can be taken directly to the Cryopreservation and Stem Cell Lab, located directly across the corridor from the Gene Therapy Lab. Entry into the Gene Therapy Lab is through an anteroom which contains personal protection items (gloves, coats, etc.) and a sink with foot pedals and electronic-sensor water flow controls. Entry from the anteroom is by card-key access only. Once within the lab, samples are taken to the assigned tissue culture bay and will remain in that area until they are to be returned to patient, put into long-term storage, or used for analysis. There is a dedicated incubator for each patient, which is decontaminated between uses, as well as scheduled LAF hood time, dedicated supplies, and equipment such as pipettors. There are also established handling procedures to ensure the safety/integrity of the materials while in the lab. For example, each patient's cells are assigned to a single incubator, media and supplies are set aside for each and are not to be used with others, only a portion of each patient's material is ever removed from the incubator at one time (if an emergency or accident should occur, not all material is lost), and a redundant number/color code system is used for labeling.

The return of material to the patient is similar to the first step of bringing it to the lab. This must be coordinated with the BMTU staff who will supervise the process as well as with the Cryopreservation and Stem Cell Lab staff. The follow-up analysis of material from the recipient/patient must also be coordinated in order for scheduling of the assays to be performed.

FLEXIBILITY AND CONVENIENCE

In designing this type of laboratory one must be able to handle the existing gene therapy protocols but also have the option of performing new ones. Flexibility is also a consideration since more than one protocol may be approved for this site, but the patient enrollment on each may vary during the course of the year. Flexibility is also a consideration when planning for possible future changes in regulations, whether they result in increased or decreased safety standards. This facility was designed so that the additional walls or doors required to upgrade the containment level could quickly and easily be added without interruption of lab functions. These include doors in the suite corridor which would access the showers and autoclave room directly into the Gene Therapy Lab and optional partitions to isolate the protocol bay from the rest of the lab. In the meantime, we have spared the expense and left the different configuration options available.

Inside the Gene Therapy Lab we have set up the two issue culture bays differently; one has a single 6 foot laminar flow hood, the other two smaller hoods. This permits assigning a single hood to a particular purpose long term and still using the area for other protocols. The laboratory furniture is suspended from a central support, and the floor is continued several inches up the support with all seams sealed in order to permit thorough cleaning underneath, and this design also permits rearrangement of the casework modules (produced by MilCare Corp., Zeeland, MI).

SAFETY ISSUES

The issues of safety in any protocol should be addressed earlier than the laboratory setup and design stages. Fundamental study design, patient selection criteria, gene vector construction, and patient management all impact on the safety as well as the efficacy of research protocols.

The concerns for the safety of the workers in a laboratory as well as the safety of the public must always be part of planning any laboratory; these are covered by local ordinances as well as OSHA and will not be discussed here. Additionally, we must also be concerned about the safety of the patient, or, in this case, the cells from the patient, while they are in the laboratory. There are several sets of guidelines which must be considered. The *NIH Guidelines for Recombinant DNA* are concerned with protecting the public and the lab workers (published in the Federal Register, section 51). The RAC (Recombinant DNA Advisory Committee) of NIH is the current forum for petition and approval of clinical gene therapy protocols, and adherence to these guidelines is mandatory. However, these guidelines are not thorough or sufficient when the considerations of patient-cell manipulations are considered. Additional information can be obtained from the *Code of Federal Regulation (CFR) 21 Parts 600–799 and Parts 800–1299* (see Federal Register) which deal with biologics, medical devices, and good manufacturing practices (GMPs) for pharmaceuticals. Other sources of information to be consulted include the institutional Infection Control Office, the Biosafety Office, Internal Review Board (IRB), and of course the Institutional Biosafety Committee (IBC) which is directly answerable to NIH on issues involving recombinant DNA. These offices will need to be satisfied before gaining institutional approval for any protocol, so an up-front consultation of individuals from each area will expedite the protocol approval process. Other recommended reading includes *Biosafety in Microbiological and Biomedical Laboratories* [US Department of Health and Human Services, 1988] and *Biosafety in the Laboratory: Prudent Practices for the Handling and Disposal of Infectious Materials* [National Research Council, 1989].

After meeting with the local officials and digesting all available regulatory materials—both required and suggested—we realized that in order to build a clinical gene therapy laboratory which met the required and anticipated standards we would need to be creative and flexible. Conceptually there are conflicting interests and occasionally conflicting regulations. On the one hand we must protect the public from potentially infectious/hazardous materials (i.e., the product), but on the other hand we must protect

the product from possible contamination from the outside (i.e., the public). Good laboratory practices and a well-planned work space will usually accomplish both; our only real problem was in the design of the air system for the suite of laboratories. A work area which is negatively pressured with respect to the outside (public corridor) is recommended in order to keep anything from getting out; however, a positively pressured room receiving only filtered air is cleaner and would reduce the chances of outside contaminants entering the area where the product is handled. In order to accomplish both objectives, we designed a system which has an exhaust sink in the ceiling of the anteroom (see Fig. 1) so neither is outside air entering the lab nor lab air escaping into the hallway. All the exhaust air from the lab leaves via the 100% exhaust laminar air flow (LAF hoods) biosafety cabinets (Forma models 1148 and 1168, Marietta, OH). Each of the LAF hoods has an air flow alarm and an airtight shutoff damper for servicing. The HEPA filters for the hood exhaust are located in a mechanical closet outside the lab so that service personnel do not need to enter the lab for filter changes.

Other basic safety considerations are covered by thorough training of personnel and regular training reviews and updates, security-card access to the interior lab from the anteroom, and standard protective outer clothing (gloves, gowns, etc.). Measures have been taken to reduce traffic and the presence of potential contamination sources. For example, the locating of gas and liquid nitrogen tanks outside the lab eliminates the need to clean off the tanks when they arrive (usually on the back of an uncovered truck) before bringing them into the lab. Supplies are unpacked from the cardboard cartons before being brought into the lab, and an outside room has been set aside for storage of bulk supplies. Cardboard boxes which have been in shipping transit or warehouse storage are not only a source of dust/dirt but potentially insect eggs and mold spores.

Some specific lab design features to note (see Fig. 1) are as follows: 1) a pass-through window for waste, which eliminates the need to carry trash out the door through which people enter and exit; 2) modular, adjustable lab casework which is suspended from a central support for easy cleaning underneath, the drawers being seamless and easily decontaminated or replaced; 3) a seamless floor that extends several inches up the wall on all sides; 4) enclosed storage areas and all nonporous surfaces for easy disinfection; 5) nonporous ceiling material; 6) showers and lockers on site; 7) a communication system including intercom and fax machines inside the lab and in the outside office (paperwork can be faxed out to the office or up to the BMTU); 8) the exterior location of all incubator gases and liquid nitrogen storage tanks in a service closet in the corridor, with even the autoclave and washers being serviced from the corridor; and 9) a fire alarm system designed to shut down the exhaust air system, with an override and warning alarm. The air system for the Gene Therapy Lab is exhausted through the LAF hoods, which provides a good, clean working environment, but in the case of a fire this is a potentially hazardous situation. A special fire alarm arrangement has been designed for this room which includes two smoke/heat detectors and a panic override button. In case of a fire in the room, the fans powering the air system will automatically shut off; if no one is in the room (and therefore no cells are being handled under the LAF hoods, etc.) this is not a problem. However, if there is material out of incubators being manipulated in the LAF hoods, an air shutoff could be a disaster. So when the first smoke/heat detector is activated, an audible-only alarm will sound, giving workers an opportunity to hit the override button (located at eye level next to the door) and investigate for a period of 2 minutes (which can be altered as needed); a call to systems engineering will abrogate the air shutoff if indeed this is a false alarm created by misuse of a hot plate or microwave. If not, cells can be safely placed in incubators and the lab will be evacuated. Any alarm in the area will eventually shut down the air system, so lab general safety procedures for fire emergency include the immediate evacuation of the area.

SUMMARY

Our intention here is to review the issues we faced during planning and design of our clinical gene therapy facility in order to assist others embarking on similar endeavors. We would be very interested in learning how others are meeting the needs of this developing technology within their clinical settings. In doing so, we

have shared the conceptual as well as technological considerations that have served as our guidelines. It should be emphasized that we have attempted to maximize organizational and operational features to support the requirements for quality control. At the same time, we have been guided by the objective of maximizing opportunities for the translation of fundamental regulatory mechanisms into gene therapy–based clinical applications.

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