

A detailed microscopic image of plant tissue, likely a cross-section of a leaf or stem, showing various cellular structures and vascular bundles. The image is rendered in a warm, golden-yellow color palette, highlighting the intricate patterns of the biological tissue.

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Dendritic Cell Vaccine Production Facility: From Design to Operation

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Melanoma is a common and deadly form of skin cancer. The American Cancer Society has estimated that there will be 62,480 new cases and 8,420 deaths from melanoma in the United States in 2008.¹

Since 1999, the Baylor Institute for Immunology Research (BIIR) in Dallas, Texas has carried out six melanoma vaccine clinical trials. These trials are conducted under Investigational New Drug Applications (INDs) filed with the FDA and are paving the way toward the development of a potential cell-based vaccine therapy for melanoma patients.

Development of a Frozen Vaccine Against Melanoma Skin Cancer

Patients with advanced melanoma cannot eliminate their cancer cells, oftentimes due to their immune systems becoming tolerant to the cancer cells. Our strategy is to educate a patient's immune system to direct cytotoxic T-cells to eliminate the melanoma cells. To accomplish this, a patient's own dendritic cell progenitors (or precursors) are

placed in cell culture. Differentiated dendritic cells are a type of white blood cell that induces and regulates immune responses (Figure 1). They are loaded with melanoma antigens (proteins unique to the cancer cells) for injection as a vaccine back into the patient. A patient receives several injections of the antigen-loaded dendritic cell vaccine.

Our clinical results have shown that this vaccine therapy is safe and well-tolerated by the patients, and can lead

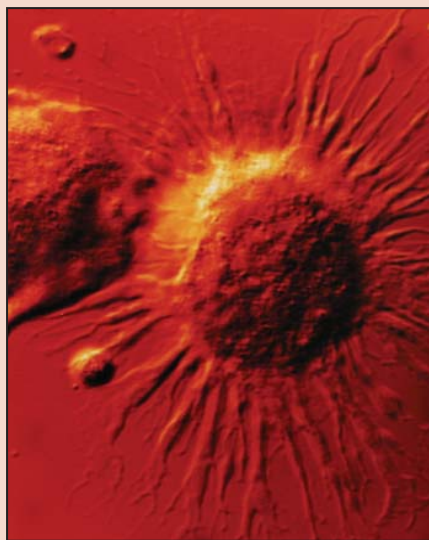


FIGURE 1. Monocyte-derived dendritic cell (white blood cell that recognizes and then binds foreign substances) used to provide a patient-specific cancer treatment.

to both elimination of the cancer and long-term survival in some patients. The ability to provide a patient-specific cancer vaccine has taken years to develop. Initially, each vaccine was manufactured fresh, which took up to nine days of preparation followed by lengthy release testing. This approach was very expensive and would have required numerous cell processing centers located in close proximity to the patient's clinic in order to provide a fresh, efficacious product. We have decreased our manufacturing time by developing new processes for the manufacture of dendritic cell vaccines.

Today, we manufacture these vaccines in three days. This allows us to produce more vaccines using the same facility and number of personnel. We have also developed a frozen dendritic cell vaccine, which enables us to ship it anywhere in the United States and possibly elsewhere in the world to the patient's local physician.

The frozen vaccine process also allows us to manufacture and release only one batch of vaccine needed for multiple injections into the patient. This has simplified and streamlined the process as well as considerably reduced the production costs.

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Dendritic Cell Vaccine Production Facility Design

In order to move product development forward, the majority of biotech companies and academic institutions involved in cell-based therapies need new facilities in order to scale up production capabilities and comply with evolving regulatory requirements.

Some institutions choose to use a contract manufacturing organization (CMO) to benefit from established expertise while others support their clinical development programs with their own dedicated production facility. The main challenges in establishing a dedicated pilot-scale production facility are described hereafter.

BIIR has used a dedicated Class 10,000 (ISO 7) cleanroom to manufacture the vaccines for clinical trials. It takes a minimum of two years for the design, fundraising, construction, and commissioning of a new production facility. Because of this, it was quite difficult to anticipate all of the future needs and the probable changes to the manufacturing process, regulatory requirements, resources, and future activity. This was like trying to solve a multi-variable equation in math with numerous unknowns.

In 2004, BIIR designed and built, with the help of Holtz BioPharma Consulting and K-Tec Cleanroom Systems, a three-cleanroom production facility able to accommodate multiple Phase II clinical trials or one large-scale Phase II/III trial (see Figures 2 and 3). The design was driven by selecting equipment (Scientific Southwest Resources, Inc.) that would accommodate most of the existing and anticipated dendritic cell vaccine processes and was flexible enough to house future changes in the required equipment.

After considering the likely vaccine production processes that rely on short-term cell culture, the decision was made to incorporate the cell culture incubators within the cleanrooms of the production suite. In contrast to this strategy, some CMOs recommend placing the cell culture incubators in a centralized location. This helps

them to minimize overloading of their production cleanrooms with various cell incubations while segregating the potential contamination risk away from the cell manipulation areas. Instead, we have integrated enough incubators in each of the cleanrooms to smoothly accommodate a seven-day manufacturing schedule with cleaning and maintenance included.

Because we use a closed cell culture system for our dendritic cell vaccine processes, cell culture media evaporation during the short-term cell incubation is not an issue. The culture bags are permeable to CO₂ but are waterproof. Our Sanyo incubators are equipped with CO₂ infrared sensors so that the incubator chambers do not require any ambient humidity from a water pan,



FIGURE 2. View of cleanroom #1 in the dendritic cell vaccine production facility at Baylor Institute for Immunology Research.



FIGURE 3. View of cleanroom #2.

thus minimizing the contamination risk. However, the incubator chambers still require extensive cleaning between patient vaccine batches even though we use a closed system. Paradoxically, the cleaning process, which involves a thorough wiping with sanitizing wet wipes, will catch most of the free particles but can displace particles from one site to another. This can cause both nonviable and viable particle counts to peak. As a result, locating the incubators and centrifuges next to the cleanroom exhaust grilles has helped control the environment by extracting particles, aerosols, humidity, or any hazardous gases from the cleanrooms (see Figure 4).

The single 6' biosafety cabinet in each of the production suite's cleanrooms is located as far as possible from the exhaust grilles to work with the room's unidirectional airflow. Our manufacturing process has a one-way flow from the introduction of the patient's apheresis blood into the cleanroom to the filling and freezing of the vaccine vials. The manufacturing personnel also have a one-way flow through the cleanrooms with separate entrance and exit doors. While the raw materials enter the cleanrooms via a pass-through window connected to other areas within the production suite, the biohazard wastes (mainly packaging materials and cell media within sealed bags) are moved out of the cleanrooms through the exit door (see Figure 5).

The two cleanrooms currently used for dendritic cell vaccine manufacture are mirrors of each other, with a common air exhaust within the middle wall. They provide redundancy to each other by having the exact same production equipment. The exhaust grilles were staggered to prevent any potential cross-contamination between the two cleanrooms. The first merging of air within the cleanroom exhausts occurs further up the wall in the plenum. A single-pass air flow allows simultaneous processes in each cleanroom without any potential cross-contamination. The room pressure, temperature, and humidity are monitored by two independent alarm systems (one monitors and controls the environment and the

second serves only as a monitor). Easy access for inspecting the air supply lines and pipes was included in the design.

In order to maximize the quality of the vaccines, every component involved in the manufacturing process is disposable and is outsourced to specialized vendors. The reagents and ancillary materials come triple-packaged, sterilized, and certified. Our quality assurance unit inspects each received lot of production materials, reviews and verifies the accompanying certificates, and releases them for use in vaccine manufacturing prior to their entering the production suite. Using disposable versus reusable materials helps the qualification of raw materials and increases the productivity of the manufacturing personnel by minimizing cleaning required between vaccine batches.

Cleanrooms and Support Area

All of the equipment and materials were carefully selected to provide ease of cleaning, durability, and adequate performance. The critical production facility is a sealed box made of modular

walls. The walls were assembled within BIIR in a period of three weeks, and an epoxy floor with 4" coves was installed. Contracting with K-Tec Cleanroom Systems enabled us to prepare the shell at the site while the modular walls were delivered and stored at their warehouse. This saved time on the overall construction schedule and it resulted in a self-contained facility isolated from the surrounding elements of the building.

Two redundant 70-ton direct-expansion (DX) units supply 7,500 cfm (60 air changes per hour of 100% outside air in a single pass). One unit is at rest while the other one is in use. They are alternated to make sure each unit has the same amount of hours on it, to change the intake pre-filters and to clean the coils. The DX unit dehumidifies, and either cools or heats the outside air prior to its introduction into the production suite. Purified steam from two redundant humidifiers is added to maintain a humidity level of around 45% within the production suite (see Figure 6). When an abnormal temperature, humidity, or pressure differential is detected, the second DX



FIGURE 4. View of cleanroom #2. Cleanroom #1 is visible through the window. Locating the incubators or a centrifuge next to the cleanroom exhaust grilles helps control the environment by extracting particles, aerosols, humidity, or any hazardous gases.

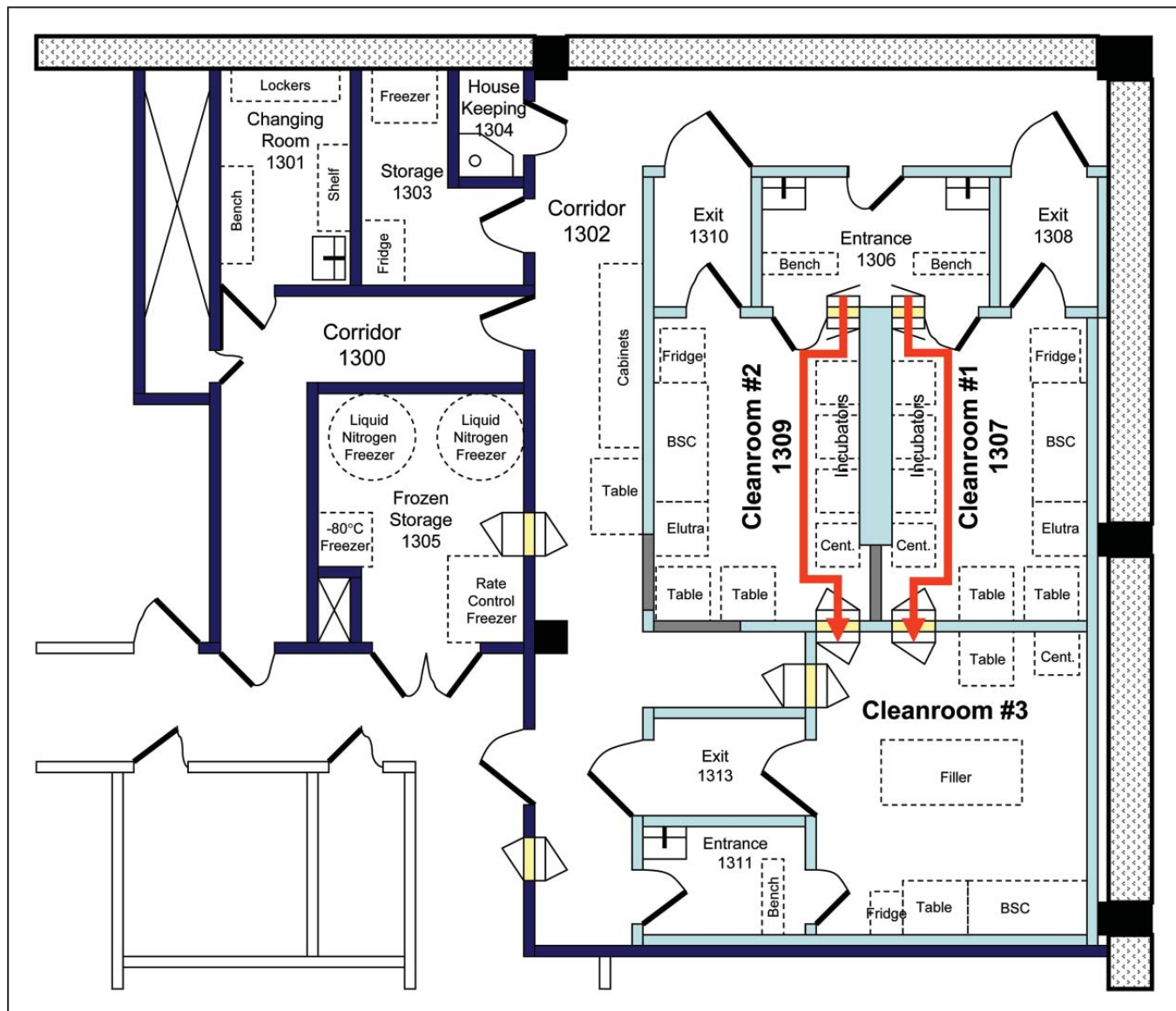


FIGURE 5 (above). Schematic diagram of the facility. The manufacturing process has a one-way flow from the apheresis to the frozen doses of vaccine. The personnel also have a one-way flow with exits distinct from entrances. While the raw materials enter the production suites via a pass-through window, the biohazard wastes move out through the exit door.



FIGURE 6 (right). Top view of mechanical support. A common supply duct enters the facility via an underground crawl space. The exhaust duct (against the building) leads to two exhaust fans. Redundant purified steam generators (which humidify the air in the facility) are visible at the top of the picture.

unit starts and, in about six minutes, reaches the proper level of cooling/heating. Automated dampers, together with the fan blower variable frequency drive (VFD), allow ramping up of the second unit as the first unit ceases operation, thus keeping a consistent airflow above 7,500 cfm that minimizes the impact of the changeover on the production suite.

The building has an electrical feed from two different transformers and an automated switch. If both transformers from the power plant happen to fail, power is provided by an emergency generator that covers the entire production suite and its support equipment, such as the DX units, exhaust fans, humidifiers, water purification system, and medical air production system. A centralized uninterrupted power supply (UPS) covers the entire production suite by buffering the electrical changes so that the computer-based equipment does not reboot. By itself, this UPS can support the entire production suite in full production for 2.5 hours if the emergency generator fails, giving us enough time to shut down the operation if necessary.

The dendritic cell vaccine process requires liquid nitrogen for freezing and storage of each patient's vaccine batch in nitrogen vapor. Two outside bulk liquid nitrogen tanks (a 3,000 gallon and a 400 gallon backup) are filled every three weeks at night by a local liquid nitrogen distributor. A 750 pound outside tank and eight backup cylinders provide CO₂ for the cell culture incubators.

Challenge 1: Implementation of Evolving Regulatory Requirements

The regulatory requirements for an early Phase I/II clinical trial are different from those for a Phase II/III trial. The early phase requirements include progressive implementation of current Good Manufacturing Practices (cGMP) and the determination of the optimal product. Total cGMP compliance and a fully-characterized product are expected for licensure in later phases of clinical and product development.

The speed of the cGMP implemen-

tation is critical. At the beginning of product development, it is important not to exhaust the limited resources by overloading them with too many operational changes. However, implementing those requirements too slowly may impact the predefined regulatory timeline.

The known requirements for most therapeutic products are listed in the current FDA Guidelines. While it may take a minimum of two years to get a new vaccine production facility constructed and operational, new regulatory requirements may arise in the meantime that can delay or significantly affect progression to commercialization of the product. Therefore, the facility design and construction project have to stay flexible to accommodate and record any changes. The justification and the review of any changes need to be tracked and well documented. Additionally, each FDA reviewer may have his or her own specific comments or questions about a manufacturing process or the production facility, which could prompt a modification. Over time, new reviewers may ask novel questions that could affect the process or operations. It is a learning exchange where the biotech or academic institution will mature its process and manufacturing competency, and the FDA will observe and regulate new emerging science and technology.

Challenge 2: Anticipation of Future Activity and Needed Resources

Starting one or several clinical trials takes time. The development of the process, preparations for the IND, the initiation of the clinical sites, and the accrual of patients require considerable effort by several individuals. As it can take six to eight months to fully train and qualify our key manufacturing personnel, this coordination can be very challenging. The recruitment of appropriate personnel can also be difficult in a small market. Vacations or illness of strategic personnel can also have a dramatic impact on the manufacturing schedule. A projection of growth can be difficult in the early

phases of product development. While the estimated yearly number of patients per disease is known, the potential number of patients that can be accrued at a given clinical location with competing clinical trials may be hard to determine. This aspect of early phase clinical trials is usually unpredictable. A wave of patients may be enrolled into a trial, thus requiring several shifts of work. Conversely, holidays, vacations, and illness can disrupt the manufacturing schedule by limiting the number of production personnel available at any given time. Our annual maintenance is scheduled during slow periods, but patient enrollment does not always coincide with these schedules.

Unexpected less-than-satisfactory environmental monitoring results may quarantine some equipment and impact the schedule. Or if a vendor announces a recall, discontinues a critical material, has a backorder situation, or some reagents require additional testing, the schedule for manufacturing will be altered. Dealing with simultaneous clinical trials, each with different cell incubation times, will give scheduling coordinators headaches. Planning for a worst-case scheduling scenario is a necessary exercise to balance production capacity against the need for supplying vaccines to the clinical trials. The cost of running a full-capacity facility can only be justified if the activities have been optimized.

Challenge 3: Manufacturing versus Operational Costs

Once a new facility is activated, there will always be certain fixed operational necessary costs. At our facility, these include: utilities, equipment maintenance and certification, sterile garment leasing, subcontracted sanitization, environmental monitoring, liquid nitrogen for vaccine storage, and CO₂ for our 24 incubators. During the transition phase from lab-scale to a fully GMP-qualified facility, these operating costs can be quite high. Operating expenses necessary for keeping the current facility operational are added to those that are essential for the new

facility's qualification period.

It should also be remembered that these fixed operational costs are incurred regardless of the utilization of the facility. Thus, personnel expenses must be factored in, especially for the core group of highly skilled technicians. Reducing the overall cost of operations is obviously linked to maintaining full production capacity. This can also be achieved by activating individual cleanrooms within the production suite only when needed and by optimizing the use of space towards one centralized location.

In our case, the manufacturing cost-per-patient vaccine batch varies with the process and the activity. Slow periods of patient accrual require smaller lots of high-priced raw materials, which may make it difficult to justify, considering the high overall costs of operation. On the other hand, high-production periods will require a larger inventory of stock materials. It helps in lowering expenses to order in bulk, although these materials must be purchased up front and then used as needed. While the initial expenditure will eventually average out over time, it is sometimes difficult to justify a large cash outlay. Anticipating stock quantities will remain an enduring challenge given the ambiguity of predicting production needs in early phases of clinical development.

The goal is to implement an average activity for our production facility with a consistent patient schedule to best control critical variables and expenses (e.g., garment inventory, sanitization, and environmental monitoring frequencies). By achieving this goal, it is possible to maintain a consistent operational cost and lower cost-per-vaccine batches.

To date, we have found our facility design to be appropriate to our needs. However, in 2008 as we move from supporting two clinical trials to initiating five separate clinical trials including a randomized trial in melanoma in May and a Phase II trial in HIV in October, we will have the true test of the full-scale performance, qualification and capacity of our facility.

ACKNOWLEDGEMENTS

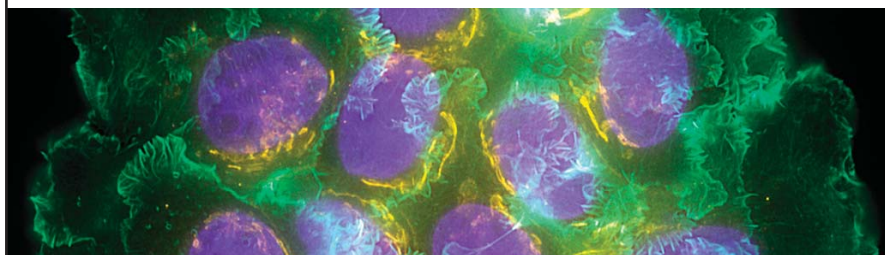
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REFERENCES

1. <http://www.cancer.org/docroot/CRI/content/CRI_2_2_1X_How_many_people_get_melanoma_skin_cancer_50.asp?nav=cri>

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Cell Culture Tip #3



Passage number counts!

Every cell line has a limit — yours does too. It's up to you to find it. Cells that are subcultured too often and are not periodically tested for genotypic stability may no longer be reliable models of the original source material.

Experimental success corresponds to the quality and condition of cell lines used. If you start to experience sudden and inexplicable variations in your experiments, it may be that the cells have lost their key functions due to over-culturing and need replacing.

REMEDY Pull a fresh vial of cells from your established cell bank or call ATCC to get a new stock. Monitor your cells routinely with morphology checks, identify markers for genes of interest and/or establish experimental criteria such as growth rates, expression levels and transfection efficiencies as baselines.

RESULTS More reliable and reproducible experiments; better data.

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