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Bioreactors get personal

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Adoptive cell transfer immunotherapy against melanoma is highly effective. However, this therapy has seen limited dissemination, mainly due to the complexity and costs of cell expansion protocols. Two bioreactors have recently been described that simplify and streamline the production of individualized cell therapies. Such bioreactors might increase the number of patients that get access to this promising therapeutic modality.

The prognosis for patients affected by metastatic melanoma remains poor, despite the recent approval by the US Food and Drug Administration of two effective therapies. Vemurafenib, an inhibitor of BRAF, most often induces short-term responses, while ipilimumab, similar to interleukin (IL)-2, is able to produce complete and durable remissions, but only in a small fraction of cases. Adoptive cell transfer (ACT) therapy can be a highly effective salvage treatment, producing durable regressions of bulky tumors in 40–72% of refractory melanoma patients.[1] ACT involves the ex vivo activation and expansion of tumor reactive T cells (tumor-infiltrating lymphocytes, TILs, or genetically engineered peripheral blood lymphocytes, PBLs), which are then reinfused (in combination with IL-2) to patients who have undergone lymphodepleting regimen.[2] Despite impressive responses, ACT is still inaccessible to most melanoma patients. This is in part due to the complicated cell expansion protocols that are currently employed, which discourage more institutions from developing ACT programs. For ACT to make the transition from a "boutique therapy" to the standard of care, the protocols for obtaining and expanding cells will have to be simplified, allowing for an efficient scale up and scale out of the process. Simplified manufacturing protocols will allow the standardization and harmonization of production across multiple institutions, constituting a critical factor for the design of appropriate Phase III clinical trials. Standardized, reliable production processes can also contribute to developing a rational, coherent regulatory foundation for individualized therapies and encourage private investors to support the commercialization of these novel and potentially curative treatments.

T-cell production for small-scale clinical trials has classically used static tissue culture systems with batch feedings. These processes are labor intensive, expensive, require frequent manipulations and do not always scale in a linear manner. Static culture systems have high volumes and are open and hence do not comply with the regulatory needs of Phase III clinical trials or commercial production. New technologies are redefining the landscape for manufacturing individualized cell therapies. With an eye on moving ACT to the next stage of development, we have identified and introduced two new bioreactors into our manufacturing facility, namely the WAVE and the newer GRex 100 bioreactor.

The WAVE is a closed system, single use, perfusion fed bioreactor that has been used extensively in the manufacture of biologics, including antibodies, and has been previously used for the production of small batches of cells for individualized cell therapy.[3] This bioreactor uses a rocking motion to generate a "wave," which creates a homogenous highly oxygenated culture environment. This, coupled with active perfusion of fresh medium, allows cells to be grown to high densities. We expanded lymphocyte cultures in the WAVE and compared them to the previous "gold standard" static gas permeable bags.[4] Compared with the static culture system, the WAVE bioreactor produced a more stable culture microenvironment. The total fold expansion of cells in the WAVE and in static bags was comparable, and functional assays demonstrated that T cells grown in these systems equivalently recognized tumor targets. Interestingly, $CD4^+$ cells preferentially expanded in the WAVE bioreactor, and $CD8^+$ cells expressed a slightly less activated/differentiated phenotype when grown in the WAVE bioreactor. Importantly, WAVE-expanded TILs were safely administered to patients and shown to be capable of mediating melanoma regression.

A second bioreactor, the Gas-permeable Rapid Expansion Flask (GRex Flask), was also evaluated at a clinical scale, in manufacturing runs for ACT.[5] The base of the GRex flask is a gas permeable membrane on which cells reside. Hence, cells are in a highly oxygenated environment, allowing them to be grown to high densities. The system scales up easily, consumes substantially less medium than the WAVE bioreactor and static bags, and requires less frequent culture manipulations. GRex flasks are compatible with standard tissue culture incubators and cellular laboratory equipment, reducing the specialized equipment and capital investment required to initiate an ACT program. GRex flasks produced comparable

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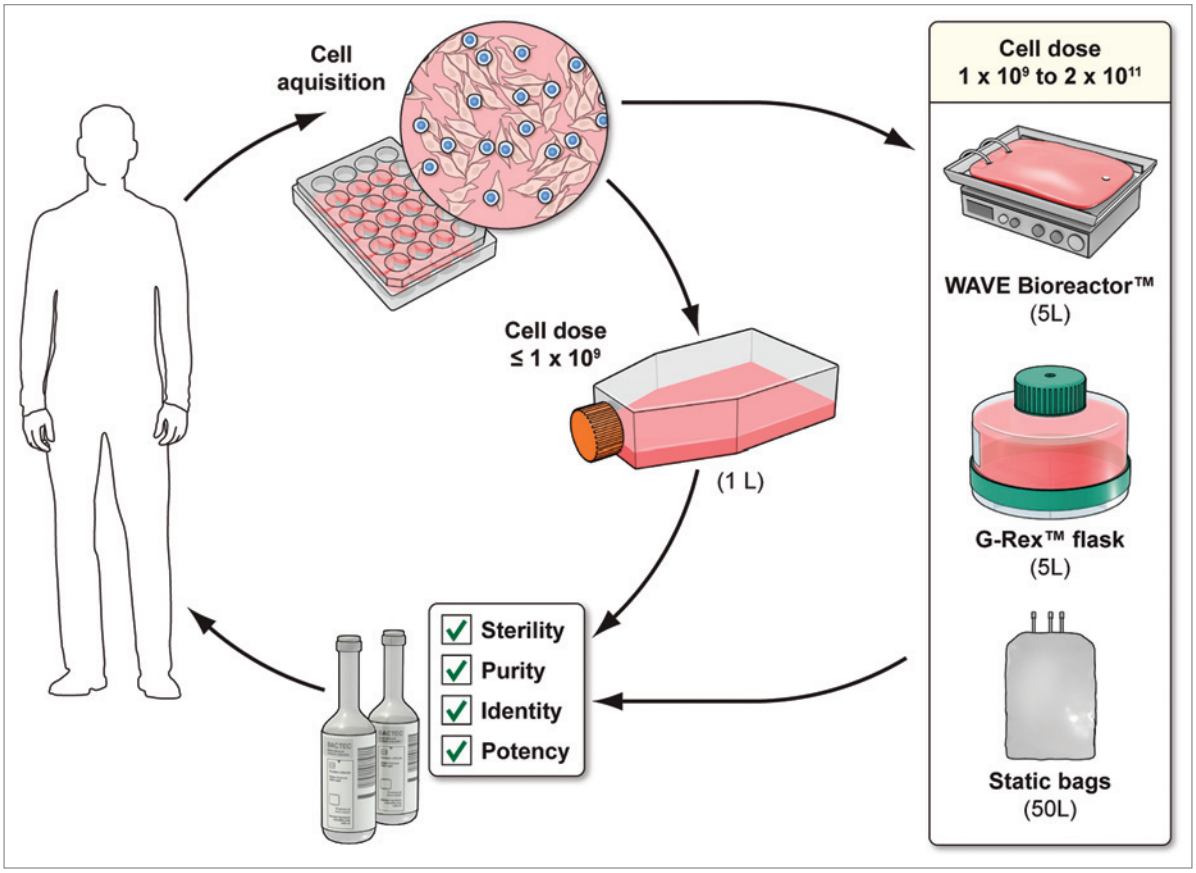

Figure 1. The manufacturing options for tumor infiltrating lymphocytes (TIL) and other individualized cell therapies proceed in three steps. First, tissue is accessioned into a laboratory and TIL cultures are initiated. Second, T cells are activated and expanded in vitro. Small scale expansions can be achieved in standard tissue culture flasks, while new bioreactors have simplified and standardized the production of the larger cell numbers that are required for some TIL therapy protocols. Finally, each product undergoes safety and efficacy testing before the final harvested product is delivered back to the patient.

or improved expansions as compared with the WAVE bioreactor as well as to the static culture systems that we had previously used. Tumor-reactive cells routinely expanded 1500 fold or more in two weeks, whether they derived from TILs or genetically modified PBLs. The phenotype and function of GRex-expanded cells was indistinguishable from those expanded in static bags. GRex bioreactor products from our lab have been safely administered to over 70 patients, and GRex-expanded TILs can mediate the regression of bulky melanomas.

The two bioreactors we describe offer substantial improvements over prior technologies. Is either device suitable for the immediate scale out and commercialization of TIL-based therapies? The WAVE bioreactor is an ideal solution for manufacturing scale up from 1 L to 1000 L, but it does not economically scale out from one patient to 1000 patients. In addition, the constant motion of the WAVE bioreactor presents challenges for process development including the first stage of manufacturing a TIL-based therapy, which requires TILs and feeder cells to remain in extended physical contact. The GRex flask is a static system that scales up and out by the simple addition of more flasks. This feature simplifies process translation from research laboratories to cGMP production and validation. However, products with large cell numbers may require up to 30 GRex flasks per patient treatment, making it challenging to test the final product for sterility, potency and cellular identity. Moreover, GRex flasks are not a completely closed system, and they do not easily integrate with upstream seeding and downstream harvesting processes. Despite these limitations, we have adopted the GRex as our primary platform for manufacturing TILs and other cell-based therapies (**Fig. 1**). Bioreactor design continues to improve rapidly, and recent prototypes from the GRex manufacturer already include larger and fully closed bioreactors.

The WAVE and GRex bioreactors represent a new generation of technology that will help deliver individualized cell therapies faster and more cheaply, by solving technical and regulatory compliance hurdles. Recent reports of impressive clinical results following ACT therapy for both solid[6,7] and hematopoietic tumors,[8,9] coupled with the simplification of cell production by bioreactors should reduce the inertia for this treatment modality and help its transition from a 'boutique therapy' to the standard of care.

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