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Manufacture of tumor- and virus-specific T lymphocytes for adoptive cell therapies

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Abstract

Adoptive transfer of tumor-infiltrating lymphocytes (TILs) and genetically engineered T lymphocytes expressing chimeric antigen receptors (CARs) or conventional alpha/beta T-cell receptors (TCRs), collectively termed adoptive cell therapy (ACT), is an emerging novel strategy to treat cancer patients. Application of ACT has been constrained by the ability to isolate and expand functional tumor-reactive T cells. The transition of ACT from a promising experimental regimen to an established standard of care treatment relies largely on the establishment of safe, efficient, robust and cost-effective cell manufacturing protocols. The manufacture of cellular products under current good manufacturing practices (cGMPs) has a critical role in the process. Herein, we review current manufacturing methods for the large-scale production of clinical-grade TILs, virus-specific and genetically modified CAR or TCR transduced T cells in the context of phase I/II clinical trials as well as the regulatory pathway to get these complex personalized cellular products to the clinic.

INTRODUCTION

Adoptive cell therapy is an emerging therapeutic platform used to induce tumor regression or clearance of certain viral infections after organ transplantation or hematopoietic stem cell transplantation (HSCT). In addition to virus-specific T cells, two major T-cell sources can confer these therapeutic properties: (1) tumor-infiltrating lymphocytes (TILs) isolated, activated and expanded *ex vivo*; (2) peripheral blood T lymphocytes engineered to express conventional alpha/beta T-cell receptors (TCRs) or tumor-recognizing chimeric antigen receptors (CARs). Clinical cell doses of these autologous tumor-reactive lymphocytes can be manufactured and infused after suitable release testing.[1–6]

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CONFLICT OF INTEREST

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The generation of clinical-grade cellular products encompasses complex processes that are tightly regulated under current good manufacturing practices (cGMP) and requires adequate cell manufacturing facility, ancillary products and manufacturing processes to meet the Food and Drug Administration (FDA) guidelines.[7] The end-of-process products characteristics such as safety, purity and potency need be carefully defined to meet the quality-control standards.[8] The manufacturing process needs to be robust and reproducible as well as cost effective. Furthermore, autologous cell therapy products are unique, and the manufacturers must integrate the scientific knowledge defining the product with the FDA regulations. In fact, the regulatory guidelines need be tailored to each individual cell therapy product. In this review, we focus on the large-scale cGMP manufacturing of cells used in adoptive cell therapy including TILs, CAR- and TCR-expressing T cells and viral-specific CTLs.

T-CELL MANUFACTURING APPROACHES

Generation of a therapeutically suitable number of highly active antitumor T cells is a significant technical challenge, and remains critical for the application of adoptive cell therapy as a standard cancer therapy.

Manufacturing of TILs

Infusion of *ex vivo*-expanded TILs has proven to be a successful treatment regiment for refractory metastatic melanoma.[9,10] The manufacture of tumor antigen-specific lymphocytes used in adoptive cell transfer is initiated from tumor fragments or single-cell enzymatic digests of resected tumor specimen. A microculture derived from a single tumor fragment or 10^6 viable cells derived from the single-cell enzymatic digestion are placed into one well of a 24-well plate with high dose interleukin-2 (IL-2). Growth medium is changed within 1 week; confluent wells are subsequently split into daughter wells and maintained as independent TIL cultures for generally 1–2 weeks. Cultures are subsequently fed twice per week and maintained at $0.8–1.6\times10^6$ mL^{-1} in flasks. A standard TIL culture typically generates 5×10^7 cells from each original well after 3 to 5 weeks of time. When tumor-reactive TIL cultures are expanded to the minimal requirement of 3×10^7 cells, independent TIL activity and specificity are determined by measuring interferon-gamma secretion by enzyme-linked immunosorbent assay post stimulation with tumor cells. Active individual TIL cultures are then expanded to therapeutic relevant numbers by using a rapid expansion protocol.[11] During the rapid expansion phase, 10^6 TIL effector cells are combined with 2×10^8 irradiated, allogeneic healthy donor peripheral blood mononuclear cell (PBMC) feeder cells in presence of anti-CD3 OKT-3 monoclonal antibody (mAb) and high dose IL-2 in tissue culture flasks. Cell density is determined on day 6 of culture and thereafter to maintain a density of 10^6 mL^{-1} by splitting TIL cultures into flasks or culture bags. IL-2 (6000 U mL^{-1}) is used throughout the process to promote cell expansion. Within 2 weeks of time since the start of the rapid expansion protocol, cells are harvested, washed, formulated and cryopreserved. The whole manufacture process takes ~6–8 weeks.[12,13] Products meeting all quality-control tests are released for patient infusion (Figure 1a).

Promising clinical outcomes have been achieved using tumor-reactive TILs in combination with lymphodepletion.[14] However, the extended duration of multiple microcultures and an individualized tumor recognition assay render the process time-consuming, complex and

costly. To circumvent the limitations of such 'standard' method, Dudley and colleagues[15] have simplified and standardized a process to culture 'young' TILs. 'Young' TIL cultures are made of bulk lymphocytes rather than individual microcultures, and the tumor recognition screening assay is eliminated from the process. Young TIL culture is initiated from enzymatic digestion of resected tumor specimen. Single-cell suspension is plated in individual wells of 24-well plates at 5×10^5 cells mL^{-1} in presence of 6000 IU mL^{-1} of IL-2. Five days after initiation, cells are fed and culture media is replaced every 2–3 days thereafter. By day 10 to 18, individual wells of cells are pooled and ~6–8 weeks.[12,13] Products meeting all quality-control tests are released for patient infusion (Figure 1a).

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Manufacturing of T cells genetically engineered to express an exogenous TCR or CAR

Despite the fact that TILs have been shown to mediate antitumor response in 50–70% of melanoma patients, TILs have only limited success in other types of cancers.[18] Furthermore, the generation of TILs is not successful for all melanoma patients.[10] To this end, the genetic

modification of peripheral blood lymphocytes to endow these readily accessible cells with antitumor activity is an attractive approach. The power and promise of TCR and CAR-T therapy have been demonstrated by encouraging outcome in patients treated with NY-ESO-1 TCR[19,20] and CD19-CAR T cells.[21–24] Many ongoing clinical trials utilized genetically modified T cells, and numerous recent papers have reported their clinical success.[25] The key requirement for this genetic modification methodology is the development of RNA vectors expressing TCRs and CARs. TCR can be cloned from the rare occurring patient tumor-reactive T-cell clones,[26] from humanized murine models[27,28] or using the phage display technology.[29,30] The design of TCR and CAR has steadily improved over the past two decades.[31–35] For CARs, tumor recognition is mediated by the single-chain variable fragment derived from a monoclonal antibody or humanized Fab. The rationale and strategy of TCR and CAR design and their evolution have been comprehensively reviewed elsewhere.[36,37]

The manufacture of T cells genetically engineered to express specific TCRs is initiated from Ficoll-purified PBMCs. T cells from PBMCs are activated with OKT-3 antibodies, transduced with a retroviral vector expressing a tumor antigen-specific TCR and cultured for ~ 2 weeks.[38] For the CAR-T cells, large-scale transduction and expansion under cGMP has been established,[39] and is also applicable to TCR-T cell manufacturing. The process is initiated from the selection and activation of T cells from patient apheresis products using Dynabeads CD3/CD28. $CD3^+CD28^+$ T cells are enriched using a magnetic particle concentrator, and are cultured at 10^6 mL^{-1}. The activated T cells are transduced with retroviral vectors in RetroNectin-coated cell bags. The retroviral vector-transduced T cells are inoculated in a WAVE bioreactor on day 6 to day 8, and expanded with a continuous perfusion regime. By the end of the production run, the beads are removed with the same magnetic bead concentrator and the cells are formulated for infusion either fresh or frozen. The process takes ~ 2 weeks (Figure 1b). This semi-closed large-scale manufacturing platform successfully supports several ongoing clinical trials at MSKCC (NCT01416974, NCT01044069, NCT00466531, NCT01840566, NCT01860937, NCT01140373)[21,23,39,40] and can be easily adapted for other clinical trials involving the transduction and expansion of autologous or donor T cells.

Other groups are focusing on defining which T-cell subsets are best suited for use in adoptive therapy to generate cell products enriched for these subsets.[41] In animal models, T-cell transfer studies have shown that effector cells from T_{EM} rapidly undergo apoptosis following adoptive transfer and do not persist beyond 7–14 days, whereas a subset of transferred $CD8^+$ $T_{E/CM}$ can reacquire memory cell markers, and persist for years.[42] Consequently, the authors developed a clinical $CD8^+$ T_{CM} purification, transduction and expansion platform that incorporates clinical scale polyclonal $CD8^+$ T_{CM} isolation from leukapheresis products, T-cell activation using anti-CD3/CD28 beads without exogenous feeder cells, lentiviral transduction and cell expansion in IL-2/IL-15.[42] This process is performed with minimal open processing steps and reproducibly yields cryopreserved cell products in excess of 10^9 cells within 35 days (Figure 1c). This platform is currently being used to generate autologous CAR redirected CD19-specific CD8+ $T_{E/CM}$ for adoptive transfer following autologous HSCT for high-risk CD19+ non-Hodgkin lymphomas.[42]

Manufacturing of viral-specific T cells (G-Rex)

Adoptive transfer of viral antigen-specific T cells is a wellestablished procedure for effective treatment of transplantassociated viral infections and virus-related malignancies. Many laboratories have successfully generated and infused T cells specific for Epstein–Barr virus (EBV), cytomegalovirus and adenovirus using monocytes and EBV-transformed lymphoblastoid cells.[43–46] Although therapeutic doses of trivirus-specific cytotoxic T lymphocytes can be generated, the original methodology requires 4 to 6 weeks of time for EBV–LCL generation and 4–8 weeks for CTL expansion.[47,48] Recent process development has been reported for the generation of penta-viral-specific T cells for CMV, AdV, EBV, BK virus (BK) and human herpes virus 6 (HHP6) with dramatically reduced production complexity and time requirement. The process starts with incubation of 1.5×10^7 fresh PBMC and overlapping 15 amino acid peptide mix spanning EBV–LMP2, BZLF1, EBNA1; Adv-Penton, Hexon; CMV-pp65, IE-1; BKV-VP1, large T; HHV6-U11, U14 and U90. The cells are subsequently transferred to G-Rex bioreactors for continuous culture in presence of IL-4 and IL-7. Therapeutic doses can be achieved in ~10 days of time, in contrast to the 10-week period required with the traditional approach. Monovalent-, bivalent-, trivalent-, tetravalent- and pentavalent-specific T-cell products are efficiently generated with this method, whereas the range of antiviral activity is limited by the previous viral exposure of the donor T cells. The multiviral-specific T-cell lines generated using this method have been demonstrated to have up to a 94% response rate in post HSCT patient with viral infections[49] (Figure 1d). Viral-specific T cells can be further genetically engineered to express TCRs and CARs to have a second specificity for tumor antigens.[44,50–52] A GMP manufacture process has also been recently tested, in which the CliniMACS-purified viral-specific T cells were transduced with retroviral vectors, and expanded *in vitro*.[53]

To broaden the use of CAR-modified T cells that could provide a GVL effect after allo-HSCT without concomitant GVHD, Riddell *et al*.[41] and others[54] have proposed to combine the use of viral-specific T cells such as CMV- and EBV-specific $CD8^+$ T cells to generate CAR expressing T cells derived from central memory T (T_{CM}) cells as they are deemed capable of persisting long term.[55] In one method, the $CD45RA^-CD8^+$ cell fraction is enriched by depletion of $CD4^+$, $CD14^+$ and $CD45RA^+$ cells on the CliniMACS device using clinical-grade mAbs and paramagnetic beads.[42] The $CD62L^+$ cells are subsequently enriched by positive selection with a clinical-grade biotin-conjugated anti-CD62L mAb and anti-biotin microbeads. In brief, the enriched $CD8^+CD62L^+$ T cells are plated with either autologous γ-irradiated peptide-pulsed PBMCs or monocyte-derived dendritic cells in 50 IU mL^{-1} IL-2. On day 1 after stimulation, the T cells are exposed to lentiviral vector stocks encoding the CD19-CAR in presence of polybrene followed by spinoculation. After 8–10 days in culture, the cells are pooled and analyzed by flow cytometry after staining with virus-specific human leukocyte antigen tetramers. The transduced T cells are expanded in culture by plating with γ-irradiated LCLs and fed with 50 IU mL^{-1} IL-2. After 10–14 days of culture, cells are stained with virus-specific human leukocyte antigen tetramers and Abs specific for transduction markers. The virus-specific subset of transduced T cells is then purified using reversible class I MHC streptamers. The selected cells can also be transduced with a lentiviral vector CAR transgene modified to co-express a truncated version of the

epidermal growth factor receptor that can be detected by biotinylated anti-EGFR (Erbitux) mAb.[56–58]

EXPRESSION VECTORS FOR GENETIC MODIFICATION OF T CELLS

Three main types of gene expression vectors are currently used in clinical applications for TCR and CAR delivery in T cells. They include gamma retroviral vectors, lentiviral vectors and transposons. We will focus herein on the large-scale manufacturing platforms for these critical reagents.

Gamma-retroviral vector

Gamma-retroviral vectors were the first viral vectors used for clinical application.[59] They are still used as gene-transfer vehicles in about 20% of the current clinical trials.[60] The wide usage of gamma retroviral vectors is due to their broad cell tropism, efficient integration and stable gene expression in target cells. In addition, they can be consistently manufactured at relatively low cost. Many stable packaging cell lines, such as PA317,[61] PG13[62] and 293GP,[63] have been developed. We share with several groups the combinatorial use of the SFG vector and PG13 packaging cell line.[23,44,64–66] The clonal selection and expansion of high-titer-producer cells can yield the desired stable gamma retroviral cell clone. Subsequently, a master cell bank of the stable packaging cell clone can be generated and qualified. Large-scale manufacturing protocols have been reported from different laboratories. The manufacturing process starts from the expansion of stable producer cells in roller bottles,[67,68] 10-layer cell factories[64] or bioreactors[69] (Figure 2a). Gamma-retroviral vectors cannot be harvested longer than three consecutive days due to the relative short half-life of gamma retroviral vectors. The harvests are pooled at the end of the production run and filtered using a step-filtration step to efficiently remove packaging cell contaminants from the vector stocks.[64,67] The vector stocks are aliquoted and frozen in vials or cryobags. In the case of gamma retroviral vectors with a self-inactivating design, vector manufacturing relies on transient transfection-based techniques similar to lentiviral vector production.[70]

To release the vector stocks for clinical use, a series of biosafety testing is required, which include but are not limited to sterility on end-of-process cells (EOP) and final product vector stocks (FP), mycoplasma testing (EOP and FP), general safety (FP), transmission electron microscopy (EM) on bulk vector stocks, *in vitro* adventitious virus testing (FP) and GalV replication-competent retrovirus (GalV RCR; EOP and FP).[39] The production of gamma retroviral vectors in serum-free media or media containing serum replacement is highly desirable for clinical trials beyond phase I but remains a challenge.[71,72] Gamma-retroviral vectors have been shown to be safe in patients who received T cells genetically modified to express LNGF-R, HSV-TK, neomycin, adenosine deaminase or an anti-HIV-1 tat ribozyme. After up to 10 years follow-up, these patients have not developed any evidence of T-cell clonal expansion.[73–76]

Lentiviral vector

Lentiviral vectors have been successfully utilized to engineer hematopoietic stem cells for the treatment of adrenoleukodystropy,[77] beta-thalassemia,[78,79] Wiskott–Aldrich syndrome[80]

and metachromatic leukodystropy[81] as well as CAR T cells for hematologic diseases.[22,56,82] Similar to gamma retroviral vectors, lentiviral vectors mediate efficient gene transfer and high level of transgene expression. The commonly used VSV-G pseudo envelop also endows broad tropism. Compared with gamma retroviral vectors, lentiviral vectors display several favorable features such as the ability to transduce nondividing cells[83–85] and relative safer chromosome integration profile;[86] it should be noted that gamma retroviral vectors have not been reported to be genotoxic in terminally differentiated cells such as T lymphocytes.[73–76] Significant hurdles in production and purification processes to obtain sufficient quantities of GMP grade lentiviral vector stocks for phase I clinical trials and beyond need to be overcome. Stable producer cell lines are difficult to generate and are not widely available for lentiviral vector production.[87,88] The commonly used manufacturing platforms for the third- and fourth-generation packaging systems are based on transient transfection of three or four independent plasmids encoding gag-pol-rev, the self-inactivating transfer vector and the pseudo envelope. For the fourth-generation packaging system, the rev gene can also be encoded on a separate plasmid. HEK293 cell and its derivatives such as 293T,[89] 293E[90] are the principle cell lines used for lentiviral vector production. The calcium phosphate precipitation method is traditionally used for transfection. Another cost-effective compound, polyethylenimine, has also been qualified and used in recent years[91,92] as well as flow electroporation.[93] Other lipidbased methods are still too expensive to be used in a large-scale manufacturing setting. For large-scale lentiviral vector production, HEK293-derived cells are expanded in large quantity. The method of culture expansion is a critical component for generating vector stocks with high titer and yield. The available scalable expansion systems include the cell factory system, the HYPERFlask, microcarriers and bioreactors.[70,94,95] The downstream processes for lentiviral vector production aims at removing cell and plasmid contaminants, concentrating vector particles to achieve high titer vector stocks while maintaining vector potency. These are challenging tasks that typically encompass the following steps: (1) Vector stocks harvesting. Owing to the nature of transient transfection, crude lentiviral vector stocks can be harvested for 2 days. Generally, the titer of the vector stocks beyond 2-day harvest is too low to be used; (2) Clarification. This step is to eliminate producer cells and cell debris from the crude harvest. It can be achieved by centrifugation or dead-end filtration. Microfiltration is needed to achieve greater clarification for downstream ultrafiltration or chromatography; (3) Nucleic acid digestion. Plasmid DNAs used for transfection are the major source of DNA contaminants. Cellular DNA and RNA may also be released during cell culture. Nucleic acids need to be removed to meet safety requirements and decrease sample viscosity, a major cause of column clotting. Benzonase is commonly used for this purpose; (4) Concentration and purification. Ultracentrifugation is the most widely used method for lentiviral vector concentration in a research setting. Ultrafiltration and chromatography are the preferred methods for manufacturing under cGMP. Although different filtration modes and devices are available for ultrafiltration, tangential-flow filtration is the most widely used method for its effectiveness and better yield. Chromatography is another preferred method for GMP manufacturing. A number of chromatography methods, including anion exchange chromatography, affinity chromatography and size exclusion chromatography[96] have been reported for the purification of lentiviral vector particles; (5) Sterile filtration and storage. Membrane filtration through 0.22 μm pores is the last step in the generation of clinicalgrade

lentiviral vector (Figure 2b). Vectors are packaged and stored in −80°C and a series of quality-control assays are performed before release for clinical use (Table 1). Similar to gamma retroviral vectors, the production in serum-free media is desirable for clinical trials beyond phase I but remains challenging.[97] The production of lentiviral vectors has been comprehensively reviewed by Schweizer and Merten.[98]

Sleeping beauty transposon/transposase system

Transposon/transposase is a relatively new expression system in the gene therapy field. It is a nonviral, plasmid-based methodology. The transposon/transposase system is derived from fish and has been adapted for gene therapy. The sleeping beauty (SB) system consists of two DNA plasmids: one plasmid is the transposon that encodes the gene of interest, such as CAR or TCR; the second plasmid expresses the transposase that enables the insertion of the transgene into TA dinucleotide repeats. The SB transposon/transposase have been used to produce genetically modified CAR-T cells for phase I/II clinical trial,[99,100] in which SB transposon/transposase are introduced into T cells by electroporation. Transfected T cells are subsequently expanded on artificial antigen-presenting cells.[101] The advantages of using the SB system are that the clinical-grade plasmids are much simpler to produce, and the cost effectiveness due to lesser safety testing requirements when compared to cell products genetically modified with gamma retroviral or lentiviral vectors (Table 2).

PROCESS VALIDATION

The bench-to-bedside transition for innovative adaptive cell therapy requires carefully designed scale-up and validation processes. Process validation is required to establish scientific evidence that a process is capable of consistently delivering quality products. FDA issued new guidelines for process validation in 2011.[102] Process validation can be broken down into the following three stages.

Process design stage

Process design is based on the knowledge gained through process development and scale-up activities, including those gained from research laboratories, process engineering, pilot and small-scale studies. The goal of this stage is to design a process suitable for routine manufacturing procedures. Early process design experiments do not need to be performed under cGMP; however, maintaining detailed records of reagents and procedures is highly advisable. During this stage, maintaining the right balance between process complexity and practicality is important to ensure broad downstream application. The selection of reagents that allows freedom to operate can be a challenge as these therapies demonstrate promising outcomes.

Process qualification stage

During this stage, the process design is evaluated to determine whether it is performing in the intended manner. This stage includes two elements: (1) Facility design as well as equipment and utility qualification; and (2) process performance qualification. Current GMP-compliant procedures must be followed at this stage.[103] Equipment and utility qualification can either be performed under individual plans or as part of an overall project

plan. The quality-control unit must review and approve the qualification plan and report. The process performance qualification combines the actual facility, utilities, equipment and trained personnel with the manufacturing process. A written protocol specifying the manufacturing conditions, controls, testing and expected outcome is essential at this stage. In most cases, process performance qualifications have a higher level of sampling, additional testing and greater scrutiny. A performance analysis report should be prepared in a timely fashion post completion of the process. The successful execution of process performance qualification is a major step in the product life cycle.

Continued process verification

This stage of validation ensures that the manufacturing process remains in a state of control. The equipment and facility qualification status must be maintained through routine monitoring, maintenance and calibration procedures.

Data generated during processes related to product quality need to be collected and analyzed in a timely fashion by qualified individuals. These results help the manufacturers gain deeper understanding of the source of variability, the presence and degree of variation and the impact of these variations on the process and product. A change /optimization of the process may be warranted based on the data collected.

Documentation at all stages of process validation life cycle is essential for effective communication in the complicated, lengthy, and multidisciplinary process of cell manufacturing. Quality is built in the product, not solely tested in the final product. An open and ongoing dialogue between manufacturing team and quality assurance/quality control teams is the key for establishing a successful manufacturing platform.

RELEASE TESTING AND CERTIFICATE OF ANALYSIS

An appropriate set of practical and scientifically defendable release criteria is essential to guarantee the products' integrity, consistency and efficacy. The underlying principle for release criteria is to provide adequate testing to ensure the product identity, purity, safety and potency. The cellular identity of T-cell products is commonly characterized by cell surface marker expression detected by flow cytometry analysis. It can also include more defined cell subset composition[42] or residual tumor contamination. The interpretation of purity here means lack of endotoxin or other potential harmful materials contaminating the product during manufacturing. Safety of the product requires that it is sterile and free of mycoplasma contamination and of RCR or RCL. Cellular products need to be viable (generally $\geqslant$ 70%), and genetically modified cellular products may need to reach a minimum transduction efficiency as a potency criteria.

Sterility is a fundamental test required for the release of cellular products. Standard sterility tests described in 21CFR610.2 for bacterial and fungal contamination requires 14 days of incubation. Bactec automated-based method are also being considered and can be validated for cultured cell products.[104,105] When only short time intervals are foreseeable between completion of manufacturing and product release, Gram staining can be used in combination with sterility results on in process samples collected 24 and 48 h before formulation. 'Points

to Consider' is the method recommended by the FDA for mycoplasma testing for all *ex vivo* cultures. Although there are other commercially available PCR-based kits to detect this contaminant, these methods are not approved by the regulatory agency. They may be used if they are properly validated during the process validation. A rapid release assay for endotoxin has been developed using the Endosafe PTS endotoxin device, which takes about 20 min and is approved by the FDA.[106] Viability assessment of cells is a routine requirement that can be done by various methods, including trypan blue exclusion, 7-aminoactinomycin D staining coupled with flow cytometric analysis, and acridine orange and propidium iodide staining followed by automatic cell counting. Other required product-specific assays should be established earlier on in the process development phase and approved by FDA under the investigational new drug application.

The release of the cell product for infusion is handled through the issuance of a certificate of analysis (C of A). The C of A summarizes the characteristics of the product and the tests performed. The C of A also details the release specifications and results of each test including the method used, assay sensitivity and acceptable range of results. Example of released tests used for CAR-T cells were previously published by our group[39] and by others.[82,101]

CONCLUSION

Treating cancers by harnessing the power of the immune system holds great promise for future cancer therapies. Cumulative evidence shows that adoptive T-cell therapy is an effective treatment for various tumors, including melanoma, hematological cancers and some viral infections post organ transplantation and HSCT. Yet, breakthroughs are still awaited in the field of solid tumors. TILs and the genetically modified TCR and CAR transduced T cells as therapeutic modalities are progressing toward a more mature stage. Although autologous cell therapy poses unprecedented challenges in terms of manufacturing and distribution for commercialization purposes, TCR- and CAR-transduced T cells recently became part of the portfolio of biotechnology and large pharmaceutical companies. Multiple partnerships between academic centers and industry have been established.[107] As a result, improved and semi-automated manufacturing platforms are likely to be developed that will allow wide dissemination of these promising therapies and will encourage novel research approaches. Adoptive T-cell therapies are poised to become part of the standard of care treatments for patients with cancer.

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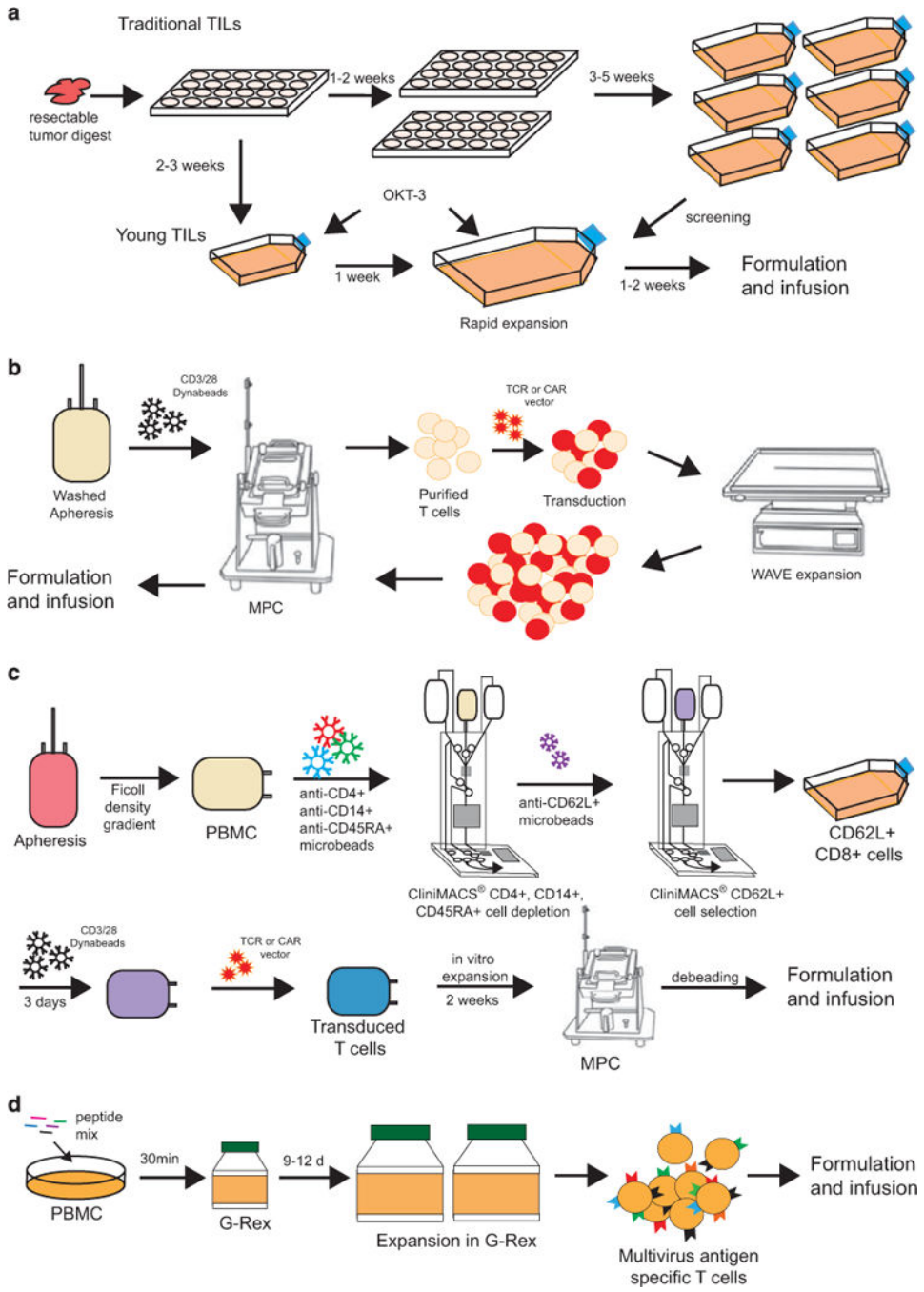

Figure 1.

Schematic illustration of representative TIL, TCR/CAR-T, and EBV-CTL manufacturing platforms. **(a)** Traditional and young TIL manufacturing scheme. Single-cell digests of resected tumor are plated in 24-well plates as microcultures. For traditional TILs, the microcultures are passaged as independent cultures. Screened and selected TIL cultures are further expanded with a 2-week rapid expansion procedure using OKT-3 antibody. For young TILs, microcultures are pooled without screening. The pooled cells undergo the same 2-week rapid expansion procedure to reach the target dose. During the whole process, cells

are maintained in culture with 6000 IU mL^{-1} of IL-2. (**b**) TCR/CAR-T manufacturing process. T cells are selected from washed apheresis product and activated by using CD3/28 Dynabeads and ClinExVivo magnetic particle concentrator (MPC). Activated T cells are transduced with TCR/CAR retroviral or lentiviral vectors, and transduced cells are expanded with WAVE bioreactor. CD3/28 magnetic Dynabeads are removed from the cells with MPC and end of the process cells are formulated for infusion. (**c**) $CD8^+$ central memory TCR/CAR T-cell manufacturing process. PBMCs are first purified from apheresis product using Ficoll-Plaque gradient centrifugation, followed by $CD4^+$, $CD14^+$ and $CD45RA^+$ cell depletion using anti-CD4, anti-CD14 and anti-CD45RA microbeads and CliniMACS. Collected cells undergo an additional CD62L positive selection procedure using anti-CD62L microbeads and CliniMACS. Selected $CD8^+CD62L^+$ cells are further activated with CD3/28 Dynabeads. Activated memory $CD8^+$ cells are transduced with TCR/CAR vectors and expanded in vitro. Dynabeads are removed from the EOP cells using MCP before formulation. (**d**) Generation of multiviral antigen-specific T cells using G-Rex bioreactor. Donor PBMCs are pulsed with 15mer peptides mix spanning EBV, adenovirus, CMV, BK virus and human herpes virus antigen epitopes and transferred into G-Rex device. Multivirus antigen-specific T cells are expanded to high quantities in ~ 2 weeks of time in the presence of IL-4 and IL-7. CAR, chimeric antigen receptor; CTL, cytotoxic T lymphocytes; EBV, Epstein–Barr virus; IL, interleukin; PBMC, peripheral mononuclear blood cell; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

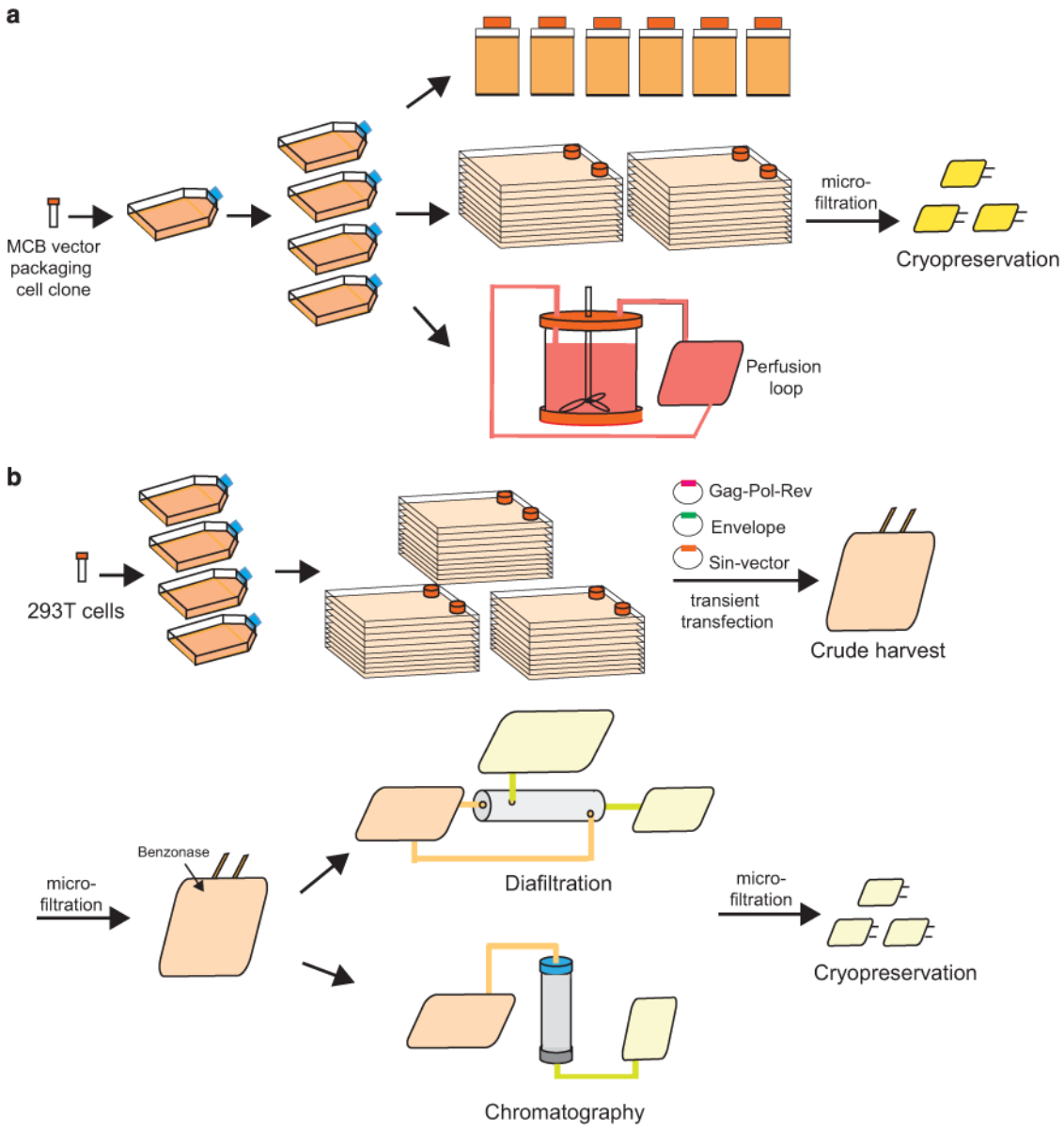

Figure 2.

Retroviral and lentiviral vectors' manufacturing platform. (**a**) Generation of retroviral vector. High titer-producer cells from master cell bank are thawed and expanded in T flasks. Cells are further expanded in either roller bottles, cell factories or bioreactors. Vector stocks are harvested in the optimized harvesting window, filtered to removed contaminants and cryopreserved for biosafety testing before release for clinical use. (**b**) Manufacturing of lentiviral vector using transient transfection in 10-layer cell factories. 293T cells or derivatives are expanded to large quantity to inoculate multiple 10-layer cell factories. Cells are transiently transfected with packaging, envelope, and SIN-vector plasmids. Crude vector stocks are harvested and filtered. Benzonase is added to the crude harvests to remove plasmid contaminants. Vector stocks need to be further purified and concentrated using diafiltration or chromatography methodologies. Purified and concentrated vector stocks are

cryopreserved for biosafety testing to be qualified for clinical use. MCB, master cell bank; SIN, self-inactivating.

Table 1

Quality-control assays for clinical-grade retroviral and lentiviral vectors

	Testing	*Example assays*	**Criteria**
Purity	Lentiviral vector		
	Total proteins (ng mL^{-1})	ELISA	Report results
	Bovine serum albumin (ng mL^{-1})	ELISA	Report results
	Benzonase (ng mL^{-1})	ELISA	Report results or <100 ng mL^{-1}
	Plasmid DNA (copies per 100 ng)	VSVg qPCR optional or serial washes	Below detection or decrease over time
	Host cell specific DNA (copies per 100 ng)	qPCR	Report results
	SV40 LTA and E1A qPCR	qPCR	Below detection
Safety	Retroviral and lentiviral vector		
	Sterility	USP, No growth for 14 days culture on Vero indicator, PTC	No growth for 14 days
	Mycoplasma		Negative
	Endotoxin/pyrogen	LAL	<10 EU mL^{-1}
	In vitro Adventitious agents	Assay on MRC5, Vero and A549 cells	Negative
	Retroviral vector		
	RCR	Marker-rescue cell culture assay	No RCR detected
	General safety (first lot)	Current USP	Absence of adverse agents
	Lentiviral vector		
	RCL	Co-culture on C8166 cells with amplification and indicator phases	No RCL detected
Potency	Retroviral and lentiviral vector		
	Infectious viral particles	Gene transfer/expression assay in cell line of choice	Report results
	Retroviral vector		
	Total viral particles	EM	Only type C retroviral like particles detected
	Lentiviral vector		
	Total viral particles	p24 ELISA, qRT-PCR	Report value
Others	Retroviral and lentiviral vector		
	Physicochemical characteristics	pH (optional)	6.9–7.8
		Appearance	Opaque

Abbreviations: ELISA, enzyme-linked immunosorbent assay; LAL, limulus amebocyte lysate; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcription PCR; RCL, replication competent lentivirus; RCR, replication competent retrovirus; USP, U.S. pharmacopeial convention.

Table 2

Example of Quality-control assays for clinical-grade SB plasmid

	Testing	*Example assays*	*Criteria*
Purity	Identity	Restriction mapping and agarose gel	Expected band size
	Sequencing	Sanger, high through put	Confirm the original coding sequence
	Concentration	absorbance at 260nm	2.0 ± 0.2 mg mL^{-1}
	A260/A280 absorbance	assay name	1.8 ± 0.2
	Plasmid Form	HPLC	>90% supercoiled
Safety	Sterility Test	USP, no growth within 14 days	No growth for 14 days
	Bacterial endotoxin	Kinetic LAL test	<50 EU mg^{-1}
	E. coli host protein	ELISA	<0.3%
	E. coli RNA	HPLC	<10%
Others	Appearance	Observation	Clear, colorless, liquid

Abbreviations: *E. coli*, *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; SB, Sleeping Beauty.