IMMUNO-ONCOLOGY INSIGHTS

ADDRESSING LINGERING SAFETY ISSUES FOR I-O PRODUCT CANDIDATES IN DEVELOPMENT

SPOTLIGHT

COMMENTARY/OPINION

Necessity for next-generation quality assessment of CAR T cell manufacturing and advanced therapy guidance

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Despite encouraging clinical results in B-cell malignancies, redirected chimeric antigen receptor (CAR) T cells bear several medical and economic challenges. On the one hand, increasing numbers of patients require reproducible and automatic manufacturing of high quality, clinical-grade CAR T cells retaining the expression of the CAR gene and their catalytic function as well as respective biomarkers to predict processing failure, which is lacking so far. On the other hand, there is an increasing interest in advanced biomarkers for therapy guidance and especially, for preclinical testing to assess side effects such as CRS and CRES.

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NEED FOR AUTOMATED CAR T CELL MANUFACTURING

The adoptive transfer of CAR T cells and the successful remissions in B cell leukemia and lymphoma is attracting growing interest for the treatment of various malignant diseases. Despite the clinical efficacy and their approval by the FDA and EMA, these patient-specific therapies must be improved regarding their robustness, reproducibility, and cost. Thus, with further applications and increasing numbers of patients, the reproducible manufacture

of high-quality clinical-grade CAR T cells in a shortened time of production is becoming an even greater challenge [1,2]. Continuous improvement has been described on the evolution of CAR design regarding increased safety, better efficacy, prolonged persistence, and effective trafficking to the cancer side [3–5]. In addition, new processing techniques, quality control mechanisms and logistic developments are required to meet both medical needs and regulatory restrictions. Still, manufacturing of autologous cells for personalized medicine is time consuming and expensive. Preliminary results with automated manufacturing gives rise to improvement in both centralized and decentralized manufacturing units [6]. However, a modular, open, and transferable system with AI-mediated robotics and digital control as well as the respective automated documentation of all in process parameters is still missing. Thus, a new concept, which addresses a 100-fold increase in number of patients if tumors can successfully be targeted is urgently needed (Figure 1).

ADVANCED CELL QUALITY ASSESSMENT TO PREDICT MANUFACTURING FAILURE

Currently, there are no harmonized rules for patient selection regarding the leukapheresis starting material and most importantly, surrogate markers are completely missing to predict production failure and functional activities of engineered T cells. In several cases, failure in manufacturing occurs because the patients are heavily pre-treated, which leads to limited bone marrow function, less functional, more exhausted T cells, and finally, a median production failure rate of approximately 7% (with a range between 1% and 17%, respectively) [7–9]. So far, it is known that steroids, the duration of pre-treatment with immune checkpoint inhibitors, ibrutinib, and immune suppressive therapies impair the quality of the leukapheresis products. This can influence the fitness of the cells substantially with a change in the senescence during the manufacturing process [10]. In addition, Marco Ruella reported on a single observation that the relapse of the disease belongs to a contaminating transduced leukemic clone with a final cis/trans formation during the manufacturing process [11]. In summary, there is an urgent need for advanced strategies to improve prediction of manufacturing failure as well as to enhance the assessment of the product after manufacturing and prior to infusion. Continuous cost reduction of genome- and transcriptome-wide methods and their unbiased assessment of cellular states facilitate identification of precise biomarkers for cell quality assessment pre- and post-CAR T cell manufacturing (Figure 1B). Next-generation sequencing (NGS) allows comprehensive characterization of genomic and transcriptomic footprints of cells, thus revealing genetic mutations or changes in pathway activities of genetically engineered T cells. Initial studies used single-cell RNA sequencing to correlate single-cell transcriptomes of CAR T cell infusion products regarding efficacy and safety [12,13]. With further studies to come, including longitudinal assessment of transcriptional variation, effects of T cell clonal diversity prior to manufacturing, or effects of the manufacturing process itself on e.g. T cell exhaustion, CAR gene transduction efficacy and cellular fitness of CAR T cells will be much better understood. The application of advanced methods (single-cell, where needed) such as NGS or Nanostring analysis will therefore be critical for the identification of novel biomarkers. These biomarkers will in turn improve pre-manufacturing cell quality assessment and thus, prediction of manufacturing failure based on investigation of the starting material, as well as improved assessment of the cell quality of the product itself.

NEED FOR ADVANCED BIOMARKER IDENTIFICATION FOR IMPROVED THERAPY GUIDANCE

Despite promising results of CAR T cell therapy, patients often relapse. This is mediated

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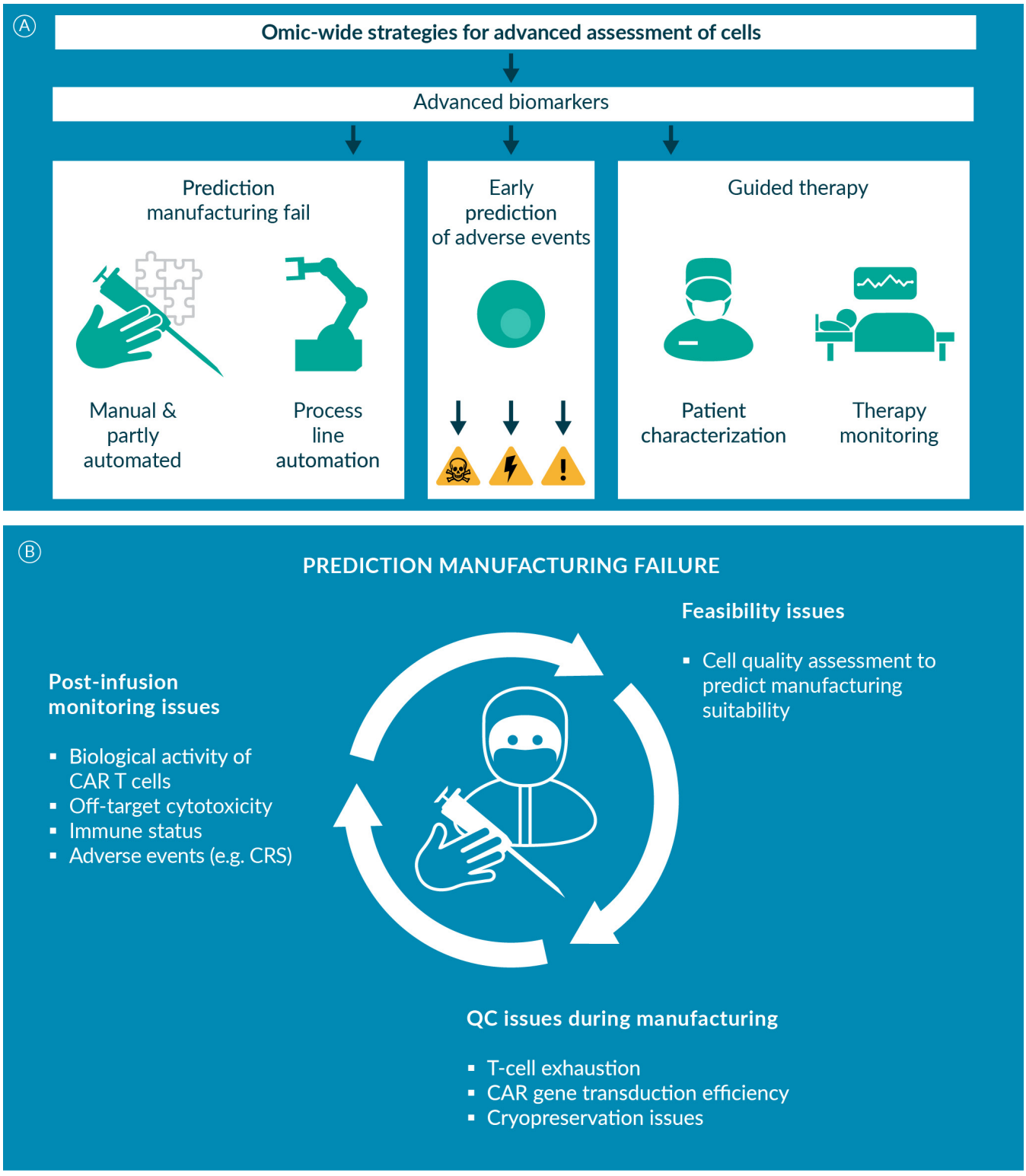
FIGURE 1

(A) Schematic overview of utilizing omic-wide strategies, such as NGS, to identify novel biomarkers, which are decisive for improvements in predicting manufacturing failure, adverse events, and therapy response of CAR T cells. (B) Representation of critical issues of CAR T cell manufacturing processes that require assessment by advanced biomarkers.



by the loss of the target structure due to selective pressure or insufficient CAR T cell persistence *in vivo* and has recently been shown to occur in an immune privileged organ, which might represent an early sign of relapse [14]. The lack of robust biomarkers predicting toxicity and/or efficacy are currently limiting the management of CAR T cells. Factors influencing the efficacy of CAR T cell therapy are highly variable and depend on the individual patients' and disease characteristics, and on the manufacturing of CAR T cell cultures (Figure 1A). Therefore, the identification of novel biomarkers predicting efficacy and toxicity, as well as early detection of relapse, are of high importance and should be implemented into the clinical routine in order to optimize CAR T cell products and the clinical benefit of this therapy. One should divide biomarkers into those predicting efficacy and those predicting toxicity, such as CRS and CRES. These are mediated by inflammatory responses and inflammation-associated tissue damages. Next to inflammatory factors, immune cells and tumor cells play vital roles in both processes.

STRATEGIES FOR ADVANCED BIOMARKER IDENTIFICATION & IMPROVED THERAPY GUIDANCE

Harmonization regarding the management of adults and children undergoing CAR T cell therapy has begun, and best practice recommendations are published from the European Society for Blood and Marrow Transplantation (EBMT) in cooperation with the Joint Accreditation Committee of International Society of Cell and Gene Therapy (ISCT) and the American Society for Transplantation and Cellular Therapy [15–17]. In contrast, less is established for guidance of CAR T cell therapy based on regular immune monitoring of patients by in depth flow cytometric characterization and advanced biomarker screening in longitudinal studies (Figure 1A). Again, genome- and transcriptome-wide strategies but also functional studies are key methods to reveal novel biomarkers to assess the individual therapy response [18]. These include T cell receptor (TCR) gene sequencing and transcriptome-wide NGS (single-cell, where needed) to analyze the CAR T cell and immune status in circulating cells (liquid biopsies) and in the case of addressing solid tumors, the tumor microenvironment. Next to NGS technologies, biomarker identification could be achieved by analysis of growth factors, cytokines, and/or chemokines in the supernatant of CAR T cells pre- and post-stimulation, and at various time points using multiplex ELISA.

In addition, CRS and CRES are key mediators of toxicities related to CAR T cell therapy. CRS results from the activation of myeloid cells by highly activated T cells and is of high interest for improved research activities. Although antibodies to the interleukin (IL)-6 receptor (e.g. tocilizumab) can ameliorate CRS, it is so far not possible to prevent CRS. In addition, factors associated with tissue damage have to be taken into account for monitoring. This gives rise to investigation and development of new biomarkers for early detection of CRS in patients' peripheral blood, as well as new models for screening mode of action (e.g. organ-on-a-chip models).

The majority of currently known biomarkers used to predict severe CRS were not detected by unbiased studies, but rather by assessing a preselected list of marker candidates [19–21]. However, utilizing unbiased approaches (e.g. NGS) for future biomarker discovery has the potential to reveal still unknown immunological characteristics leading to severe CRS and thus, to development of more precise biomarkers [13]. Currently, the EU project imSAVAR (immune safety avatar: non-clinical mimicking of the immune system effects of immunomodulatory therapies) is aiming to create a platform of novel tools, models and resources for early preclinical prediction of possible adverse events of immunomodulatory therapies. In the future, this platform should guide early preclinical safety assessment of novel immunotherapeutics, thereby reducing the cost of their development.

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CONCLUSIONS

It is noteworthy that responses to CAR T cell therapies vary considerably, which is due to the patients' and disease characteristics and procedures of the CAR T cell culture process. This might be characterized by a distinct composition of immune cell subpopulations and their function. On the one hand, this can be explored in detail by immunology-based technologies (e.g. multicolour flow cytometry or CyTOF) or by molecular biological methods (in particular, high throughput screening using NGS and/or Nanostring analysis). On the other hand, comprehensive and integrative bioinformatics analyses of the retrieved datasets linking biomarker candidates to (longitudinal) clinical outcomes in cohorts of representative sample size will be decisive for improvements in quality assessment of CAR T cell manufacturing and therapy guidance. The availability of novel biomarkers will be the key to providing critical information for the therapeutic success and failure of CAR T cell therapy, which could then be used to improve and optimize the efficacy and safety of this approach.

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AUTHORSHIP & CONFLICT OF INTEREST

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