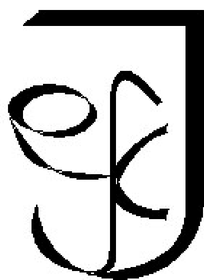

The potential of excipients to improve the efficiency of immuno-oncology therapy.

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Editorial

The success of a paradigm......is at the start, largely a promise of success....

T.S.Kuhn, The structure of scientific revolutions

ABSTRACT

The cocktail of substances used in cell culture media to cryopreserve, transfect, grow, expand, fractionate, concentrate, wash and remove impurities from leukapheresis harvested T-cells are functional excipients. Even though most of them are not present in the final product, they nonetheless have the potential – during *in vitro* manufacture - to determine the subsequent *in vivo* proliferative capacity, persistance, safety and compositional phenotype of the injected re-engineered T-cells. Thus, while the chimeric antigen receptor and co-stimulatory signaling molecules are necessary for CAR-T cell functionality, they may not be sufficient to achieve this functionality unless manufactured using the right cocktail of functional excipients.

KEY WORDS: Cell culture media, immuno-oncology, CAR-T cells, functional excipient, chimeric antigen receptor

Based on order(s)-of-magnitude increase in short-term remission rates for pediatric acute lymphoblastic leukemia (ALL), an FDA oncologic drugs advisory committee unanimously voted to recommend the approval of the first of its kind autologous chimeric antigen receptor -T cell (CAR-T) therapy. The unprecedented effectiveness of CAR-T in chemotherapy refractory patients, acting independently of major histocompatibility complex (MHC) signaling, was enough to justify this recommendation, despite significant short and long term, known and unknown, safety concerns and manufacturing issues. Much will depend upon whether this promise of success is actualized and Immuno-oncology (IO) ushers in a new paradigm in cancer cure.

There are multiple factors that may determine the effectiveness (including the proliferation and persistance) and safety of CAR-T therapy. These include: the age of the patient, exposure to prior chemotherapy before leukapheresis as well as between the vein-to-vein time period, the co-stimulatory and/or suicide molecule(s) engineered into the CAR-T cells, the number of CAR-T cells dosed, extent of immunodepletion before dosing, including the drugs used to cause such immunodepletion, bioreactor culture composition, gas perfusion and mixing conditions and growth rate to expand T-cell or

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[complete or ratio-separate] T-subcell ($CD4^+$, $CD8^+$) populations; or to polarize T-cells to a specific phenotype *ex vivo*, or to attenuate the percent of T-cells that become exhausted, the viral vector composition and sterility, DNA transduction efficiency, and the infusion medium/buffer composition of the washed and concentrated CAR-T cells. The manufacture of these products represents a formidable scientific, technical and logistical challenge.

A variety of dietary components such as dietary nucelotides and alkyl-amines, L-theanine, DHA, vitamins, fatty acids, polyphenols and probiotics, have been shown to influence T-cell homeostasis, metabolic phenotype (glycolysis *versus* mitochondrial respiration), differentiation (including T_{eff} to T_{reg} ratio and skewing the population toward a stem or central memory phenotype), cytokine secretion and immunosurveillance *ex vivo*. Glycerides, in particular, glycerol monolaurate, and the glycerides in fat emulsions used for total parenteral nutrition (TPN), have been shown to modulate T-cell function through a structural alteration of the plasma membrane, suggesting that emulsifier type and concentration used in the culture medium may affect CAR-T cell functionality. In this regard, surfactants that are non-imunogenic, such as alkylsaccharides, may prove worthy of further research. The pH of the medium during *ex vivo* expansion has been shown to affect apoptosis and interleukin receptor expression, suggesting that at least part of the cytokine release syndrome (CRS) observed upon CAR-T infusion may be attenuated by pH control during *in vitro* expansion. Since gas permeation and exchange occur continuously during manufacture, the use of buffers dependent upon the partial pressure of CO2 which are present in standard cell-culture protocols - may prove an unwise choice.

Changing the concentrations of (say) the vitamins folic acid, niacinamide or riboflavin in modified Dulbecco's medium (MDM) may not intuitively be seen to influence expanded CAR-T functionality. However, compositional variation studies in the 9 inorganic salts, 20 amino acids, 10 vitamins plus a sugar and buffer ingredient that constitute MDM have remained sparse to non-existent. This is in part because this ubiquitously used standardized 'base' reagent has acquired a unified unchangeable perception. Note that there is no recourse but to recognize the above mentioned molecules as functional excipients because they influence the clinical efficacy of the product; a proposition that the author has long been a proponent of.

A judicious utilization of these molecules, not necessarily in the concentrations present in standardized cell expansion reagents, such as MDM, during the *in vitro* CAR-T manufacturing process can modulate/optimize the potential of the engineered T-cells as well as significantly reduce the manufacturing time from weeks to days. These functional excipients that are present during manufacturing, but are not part of the infused product, may nonetheless influence CAR-T proliferation, persistance and immunosurveillance properties post infusion. On the other hand, cryopreservants such as DMSO are usually present in the infused product. It may bode well to look at this molecule as a factor linked to the cerebral edema and neurotoxicity of CAR-T therapy, in addition to, or in conjunction with, other proposed factors, such as co-stimulatory molecules and specific drugs used for immunodepletion, that are currently under consideration.

Until such time as off-the-shelf, allogenic universal CAR-T cells materialize, each batch of autologous CAR-T cells is designed for a different patient and needs to perform in tandem with the clinical status of that particular patient. Thus, while some processes such as washing and transduction can be fully automated across batches; the same level of automation may not be possible for processes which influence engineered T-cell functionality, because these are patient factor dependent. It

follows therefore, that the concentration of excipients, or indeed, even the excipient composition, may need to change from batch-to-batch; leading to a challenging regulatory landscape.

There is general agreement that IO is a rapidly evolving field and an in-depth accumulation of knowledge about the mechanism of action and safety of these 'living drugs' has not kept pace with their runaway and apparent unprecedented clinical success. Such a 'learn as you go' scenario lends further impetus to investigate the role of functional excipients to manufacture specific populations of transfected T-cells in specific ratios and phenotypes to gain clinical or competitive advantage and to expand clinical indications.