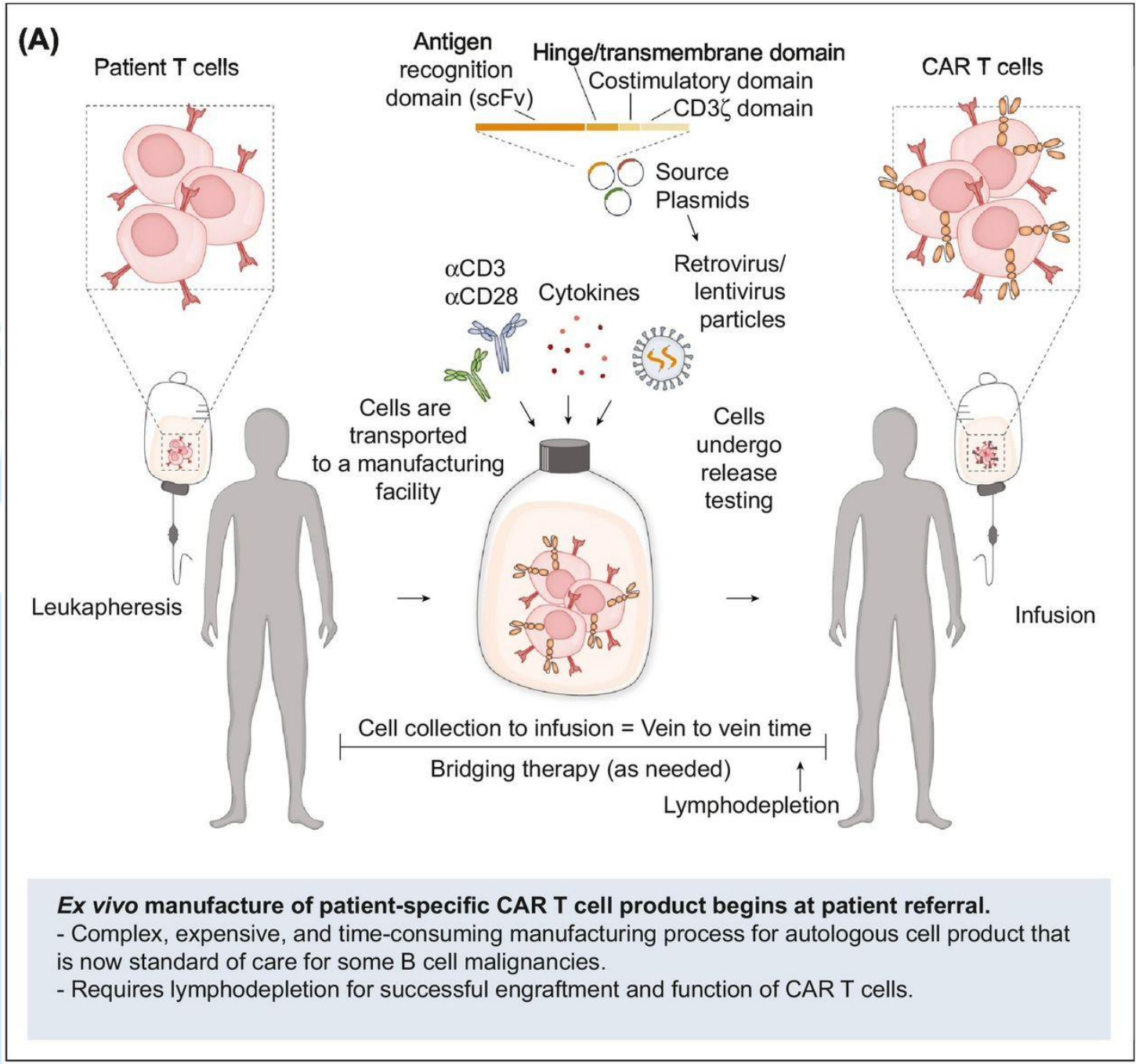

***Ex vivo* manufacture of patient-specific CAR T cell product begins at patient referral.**

- Complex, expensive, and time-consuming manufacturing process for autologous cell product that is now standard of care for some B cell malignancies.
- Requires lymphodepletion for successful engraftment and function of CAR T cells.

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Trends in
Pharmacological Sciences

Review

Direct *in vivo* CAR T cell engineering

Lauralie Short,[1,2] Robert A. Holt,[1,2,3,4] Pieter R. Cullis,[5,*] and Laura Evgin [1,2,3,*]

T cells modified to express intelligently designed chimeric antigen receptors (CARs) are exceptionally powerful therapeutic agents for relapsed and refractory blood cancers and have the potential to revolutionize therapy for many other diseases. To circumvent the complexity and cost associated with broad-scale implementation of *ex vivo* manufactured adoptive cell therapy products, alternative strategies to generate CAR T cells *in vivo* by direct infusion of nanoparticle-formulated nucleic acids or engineered viral vectors under development have received a great deal of attention in the past few years. Here, we outline the *ex vivo* manufacturing process as a motivating framework for direct *in vivo* strategies and discuss emerging data from preclinical models to highlight the potency of the *in vivo* approach, the applicability for new disease indications, and the remaining challenges associated with clinical readiness, including delivery specificity, long term efficacy, and safety.

Highlights

Adoptive cell therapy using chimeric antigen receptor (CAR) T cells is effective against B cell malignancies; however, the complex manufacturing process and financial realities constrain the scalability of the approach.

The *in vivo* generation of CAR T cells, and possibly other immune cells, using off-the-shelf products therefore has numerous logistical and functional advantages.

In preclinical models, *in vivo* gene delivery using nanoparticles or viral vectors has yielded CAR T cells with therapeutic equivalency to *ex vivo* generated CAR T cells.

Ongoing research efforts are attempting to determine how to best target and leverage effector cells of interest (T cells, macrophages etc.), understand how direct *in vivo* CAR generation interfaces with other immune cells, and optimize design elements of the viral vectors or nanoparticle and nucleic acid formulations.

Therapeutic retargeting of T cells with synthetic receptors

CAR T cell therapy has been a breakthrough approach for the treatment of relapsed/refractory (r/r) B cell malignancies. The synthetic CAR molecule fuses a targeting moiety, typically an antibody-derived **single chain variable fragment (scFv)** (see Glossary), to intracellular CD3ζ and co-stimulatory domains (such as CD28 or CD137; also known as 4-1BB) to redirect T cell function toward tumor surface-expressed antigens. Although the greatest progress has been made in the treatment of leukemia, lymphoma, and myeloma (Box 1), the modularity of the CAR molecule renders it highly versatile, and the preclinical and clinical pipeline is full of novel CAR targets and constructs for hematologic malignancies, solid tumors, and nononcology applications (i.e., autoimmune diseases, infectious diseases, etc.) [1]. Within the past 10 years, innovative new CAR designs and long-term clinical results have emerged that have cemented the therapeutic power of the synthetic immune receptor. However, this success raises the enormous challenge of how to clinically implement a bespoke *ex vivo* manufactured cell product at a broad scale for cancer indications and beyond. Here, we describe the difficulties associated with current *ex vivo* CAR T cell manufacturing practices and discuss two technological advances, nanoparticles encapsulating CAR encoding nucleic acid and viral vectors encoding the CAR, that can be infused directly into a patient to address this unmet need for a universal off-the-shelf product.

Current CAR T cell manufacturing practices

All currently approved CAR T cell products, and a majority of cell therapies undergoing clinical testing, are produced *ex vivo* from patient autologous T cells. Preparation of this bespoke treatment involves a multistep process that requires highly skilled technicians, strict quality management systems, and specialized facilities and equipment (Figure 1A) and is reviewed in [2]. Since viral transduction methods are commonly used to deliver the CAR transgene to T cells, **γ-retroviral** or **lentiviral vectors** must be manufactured by introducing source plasmids into producer cells to generate viral particles. The T cells from a patient are collected by leukapheresis, transported to a manufacturing facility, activated in culture by CD3 and CD28 co-stimulatory molecules and cytokines, transduced with virus, and expanded to yield an individualized cell

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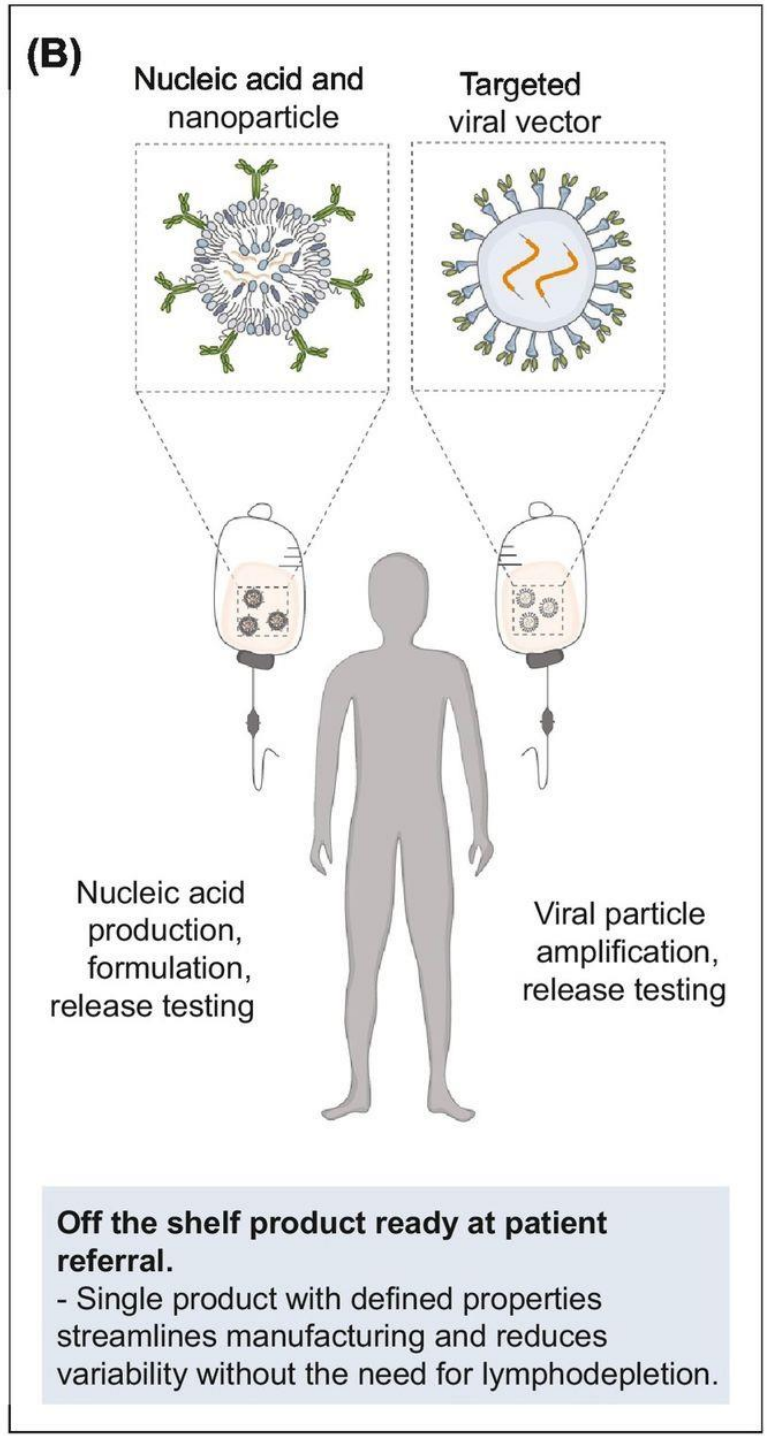
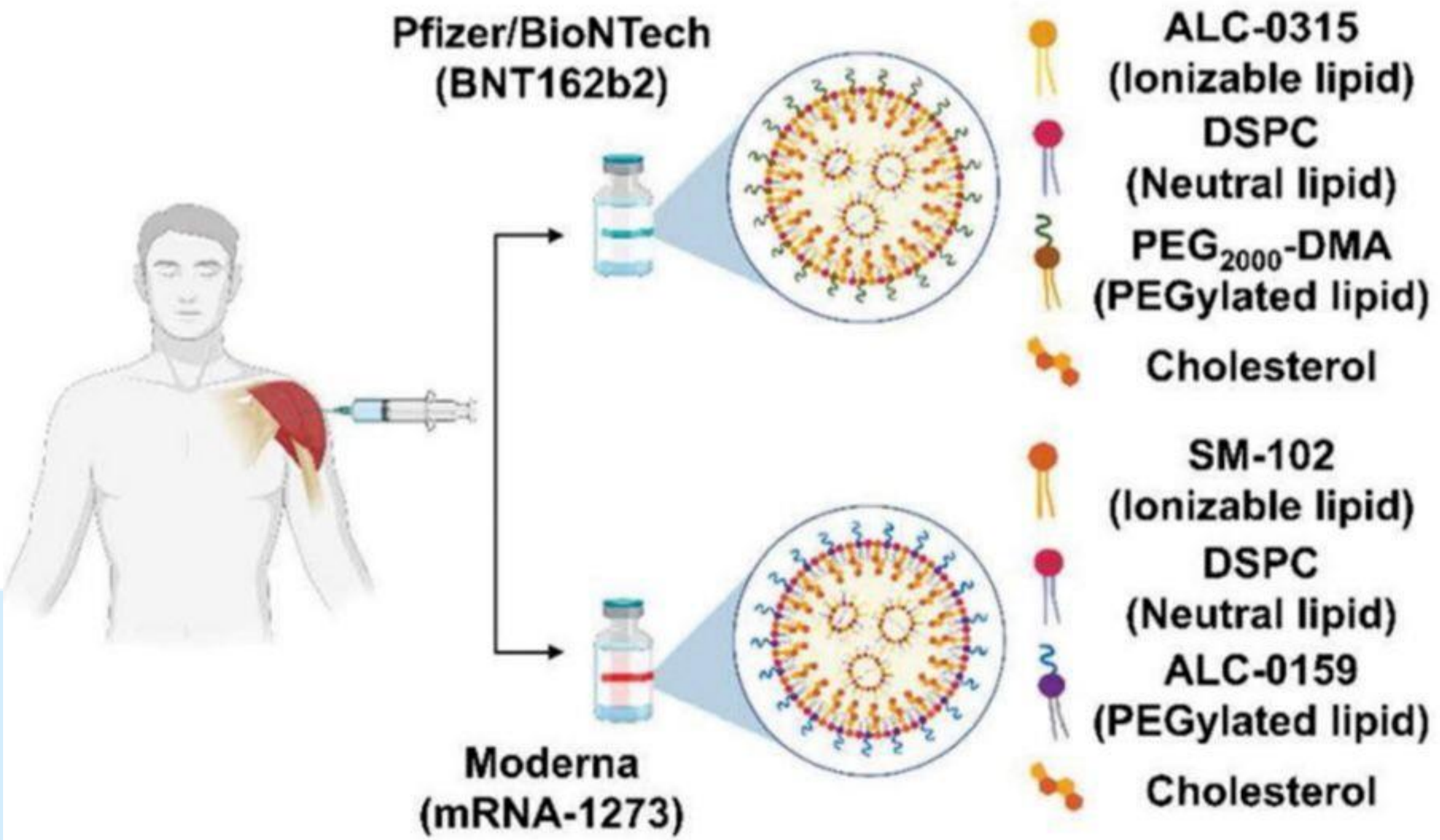

(B)
Nucleic acid and nanoparticle
Targeted viral vector
Nucleic acid production, formulation, release testing
Viral particle amplification, release testing
Off the shelf product ready at patient referral.
- Single product with defined properties streamlines manufacturing and reduces variability without the need for lymphodepletion.


Pfizer/BioNTech
(BNT162b2)
ALC-0315
(Ionizable lipid)
DSPC
(Neutral lipid)
PEG2000-DMA
(PEGylated lipid)
Cholesterol
SM-102
(Ionizable lipid)
DSPC
(Neutral lipid)
ALC-0159
(PEGylated lipid)
Cholesterol
Moderna
(mRNA-1273)

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In vivo CAR T cells move into clinical trials

Drug developers want to reprogramme immune cells directly in the body, opening up new gene therapy frontiers in cancer and autoimmunity.

By Asher Mullard