

**Correspondence on Editorial regarding “Genetically modified, redirected T cells target hepatitis B surface antigen-positive hepatocytes and hepatocellular carcinoma lesions in a clinical setting”**

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**Running title:**

Correspondence to Editorial on T-cell therapy of HBV-HCC

**Abbreviations:**

**ALT** Alanine aminotransferase

**CAR** Chimeric antigen receptor

**CLDN-6** Claudin-6  
**CRS** Cytokine release syndrome  
**DNA** Deoxyribonucleic acid  
**HBsAg** Hepatitis B virus surface antigen  
**HBV** Hepatitis B virus  
**HBV-DNA** Deoxyribonucleic acid of hepatitis B virus  
**HCC** Hepatocellular carcinoma  
**RNA** Ribonucleic acid  
**TCR** T-cell receptor  
**TCR-T** T-cell receptor T cells

**Conflict of interest:**

KW and KZ are employees of SCG Cell Therapy; KW, KZ, and UP are shareholders and directors of SCG Cell Therapy Pte. Ltd..

Accepted article

Dear Editor,

We thank Dr. Antonio Bertoletti and Dr. Anthony T. Tan for their interests in our recent publication “Genetically redirected HBV-specific T cells target HBsAg-positive hepatocytes and primary lesions in HBV-associated HCC”<sup>1</sup> and their thoughtful commentary.<sup>2</sup> We welcome the opportunity to discuss the evolving field of T-cell receptor (TCR)-T cell therapy for hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) and the potential of different strategies in this complex clinical setting. In addressing this challenge, two distinct approaches have emerged: the use of RNA-electroporated T cells with a short-term expression of the HBV-specific TCR, pioneered by Bertoletti and colleagues, and our approach involving lentiviral transduction to achieve stable TCR expression. Both strategies have been advanced in clinical settings through the biotech start-ups Lion TCR and SCG Cell Therapy, respectively. We want to take the opportunity to highlight key considerations as to why we chose stable TCR expression.

One of the key advantages of genetically modified TCR-T cell therapies, such as the lentivirally-transduced SCG101 used in our study, is their ability to establish memory T cells. This includes T memory stem cells, a rare subset of memory lymphocytes endowed with the stem cell-like ability to self-renew and the multipotent capacity to reconstitute the entire spectrum of memory and effector subsets. These memory cells are crucial for long-term immune surveillance and control of viral infections and tumors - under physiological conditions and in cell therapy.<sup>3,4</sup> We demonstrated the presence of stem-cell-like memory T-cells carrying the TCR in our patient, indicating that these TCR-T cells persist and provide long-term immune memory. This allows for a robust, durable response that is not achievable with transiently expressed TCR-T cells.

Along that line, the persistence of infused T cells is a key factor in the success of adoptive cell therapies in hematologic malignancies, and chimeric antigen receptor (CAR)-T cells from complete-responding patients with chronic lymphocytic leukemia were shown to be enriched in memory-related genes.<sup>5</sup> In solid tumors, a lack of persistence of transferred cells has been identified as one of the major challenges, and a lot of research has focused on ways to support T-cell longevity.<sup>6</sup> All these efforts centered around stably genetically modified T cells as persistence cannot be achieved with RNA-electroporated cells.

Most convincingly, clinical studies that have analyzed T-cell persistence found a positive correlation with the response to T-cell therapy. In a trial in the early days of T-cell therapy, it was observed that neuroblastoma patients with cell persistence for more than six weeks had less progression of their tumors.<sup>7</sup> More recently, it was shown that clinical responses to treatment with claudin-6 (CLDN6)-specific CAR-T cells only occurred in patients with a sustained T-cell persistence over six weeks.<sup>8</sup> Similarly, the recent clinical data from Adaptimmune's TCR-T-cell product, the world's first approved TCR-T therapy (Afamitresgene Autoleucel), demonstrated a correlation between exceptionally good T-cell persistence and overall survival in a phase II pivotal trial.<sup>9</sup> Our findings align with this perspective and suggest that TCR-T cells' durable persistence is associated with better clinical outcomes in HBV-associated HCC

(unpublished results). In the following, we summarize the arguments that convinced us to use stably transduced TCR-T cells.

1. While stating T-cell persistence is indispensable, we acknowledge the discussion regarding T-cell exhaustion and the importance of maintaining functionality over time. However, it is at least possible to maintain functionality for stably transduced TCR-T cells while they persist. In contrast, mRNA-transfected T cells rapidly lose their TCR and thus cannot maintain their specificity and functionality. Although experimental data proving T-cell functionality are unavailable for the patient described, the observation of long-term tumor and virus control indicates the long-lasting functional activity of the TCR-engineered T cells, which is consistent with its capability of forming and maintaining memory T cells.
2. In their commentary, Bertolotti and Tan raised the point that neither RNA-electroporated nor stably transduced HBV surface antigen (HBsAg)-specific TCR-T cells may distinguish between HBV-infected non-tumor cells with integrated HBV-DNA, and HCC cells. This is certainly correct, and we appreciate that they have pioneered HBV-HCC T-cell therapy with the careful approach of using RNA-electroporated T cells. While this approach did not lead to any cytokine release syndrome (CRS) development and only occasional liver function alterations, no significant on-target activity was observed.<sup>10</sup> We hence concluded that another approach is required to increase target engagement and decided to exploit stable TCR expression. This indeed resulted in a fundamental reduction of HBsAg accompanied by alanine aminotransferase (ALT) flares proving the T cells' on-target activity. Safety considerations graded the elevation of liver enzymes within the range of benign flares,<sup>11</sup> most likely crucial for achieving long-term virus and tumor control. Targeting and eradicating hepatocytes carrying integrated HBV-DNA in tumors and in non-tumor and premalignant cells is a vital component for improving overall treatment outcomes in HBV-HCC and preventing recurrence.<sup>12</sup>
3. An important consideration in the development of T-cell therapies is the development of an anti-drug immune response. Such an immune response may arise after infusion of any kind of TCR-T cells as the TCR sequence is fully human but has been derived from another individual and is foreign to the recipient. Without prior lymphodepletion, a TCR-T-cell transfer can induce anti-TCR antibodies and anti-TCR-T-cell responses at least in a xenogeneic setting.<sup>13</sup> Repeated lymphodepletion, however, is not feasible and the chance of anti-drug immunity largely increases with multiple dosing, a concept widely applied in vaccination schemes. This holds especially true for mRNA-containing products because mRNA is very potent in inducing immune responses.<sup>14</sup> Further human studies are needed to determine the extent to which these autoreactive immune responses occur after T-cell therapy and if anti-TCR antibodies can neutralize the infused cells, eliminate their effectiveness, or increase the risk of adverse reactions.
4. Treatment costs of T-cell therapies are a big issue. The persistence of lentivirally-transduced TCR-T cells and their capacity to amplify without losing their TCR requires only a single infusion, which largely reduces the time and costs for product

fill and finish, storage, and administration, reduces hospital visits and thereby provides a better cost-benefit-ratio.

We believe that the data presented in our study provide a compelling argument for the suitability of lentiviral-transduced, stable TCR-expressing T cells for treating HBV-related HCC. The ability to form memory T cells, avoid repeated infusions and potential immunogenicity of the TCR product, and achieve lasting therapeutic effects makes our approach viable and effective. We are grateful for the opportunity to contribute to this important discussion on the evolving landscape of TCR-T-cell therapy for HBV-HCC. The development of effective, safe, and long-lasting treatments remains a priority, and we believe that diverse approaches, optimizing dose regimens, and exploring novel booster strategies, hold great promise.

We are looking forward to continued advancements in the field and collaborative efforts to improve the outcomes for the huge number of patients with HBV-related HCC.

Accepted article

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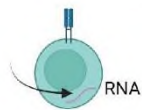
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### Transient TCR expression

#### PRO

- Higher safety because the TCR vanishes after a few days
- No genetic integration
- Low / transient CRS

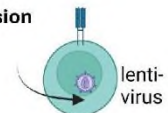
#### CON

- No T-cell persistence
- Repeated infusions needed
- Weak on-target reactivity
- High chance of anti-TCR immune responses

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### Stable TCR expression



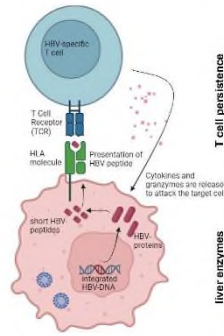
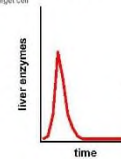
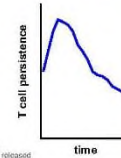
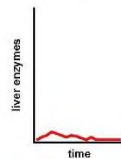
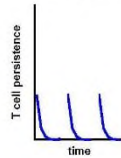
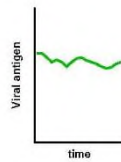
#### PRO

- Long-term T-cell persistence
- Single infusion due to cellular self-renewal
- T-cell memory formation
- Strong on-target activity
- Efficient removal of diseased cells

#### CON

- Genetic integration
- Transient, benign liver flare
- Triggering of the patient's immune system and CRS

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