Accelerating *In Vivo* CAR T-cell Development

Dr. Raymond Schiffelers
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Innovation: Developing novel delivery approaches for creating CAR T cells *in vivo*, including non-viral delivery combined with DNA- and RNA-transposase technologies

Challenge: Detecting cargo, delivery, integration, and translation to improve screening efficiency and optimize construct design

Barcoding Solution: Rapidly and cost-effectively track delivery and translation of multiple LNP and CAR constructs, accelerating discovery

Advantages: Protein barcoding on Platinum provides an easy-to-use, accessible alternative to mass spectrometry, facilitating efficient tracking of functional delivery of different vehicles

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We recently spoke with Dr. Raymond Schiffelers, Professor at UMC Utrecht and NanoCell TX's Vice President of Preclinical Research and Development, about the need to accelerate *in vivo* CAR T-cell development to enable efficient tracking of functional delivery, optimizing construct design and screen efficiency.

Q: Tell us about the work you are doing.

A: We are trying to develop a nanoparticle that can transform T cells *in vivo* to CAR T cells. So no longer the *ex vivo* process but instead targeting the T cells *in vivo* and transforming them into T cells that can target tumors. To do that, you must make multiple choices: you need to choose the best targeting ligand on the surface, the composition of your nanoparticle, the ratio between the messenger RNA, DNA, whatever you want to put in. These experiments can rapidly get out of hand because you cannot optimize one single variable at a time you have to do them in a sort of matrix of different choices that you can make, different compositions, different targeting ligands, different densities of targeting ligands on the surface. This can rapidly escalate into thousands and thousands of different particles that you could potentially test, which is of course impossible, especially if you don't do it in a barcoded way.

Q: What are the challenges that led you to explore protein barcoding and sequencing with the Platinum instrument?

A: Up to now, people have been using messenger RNA or DNA barcodes to label different particles. But then you can only look at localization so, does it arrive in the correct cell or in the vicinity of the correct cell or correct tissue? The problem is that it does not tell you about the success of delivery. So much can go wrong even if you arrive in the right tissue in the right cell, it just sticks there in the endosomal lysosomal compartment and nothing happens, then you get 100% targeting efficiency, but there's no expression whatsoever. To be able to track successful delivery and translation, and to do that in a barcoded fashion, is the biggest advantage that Quantum-Si's protein barcoding offers.

Q: Have you considered using mass spectrometry-based protein barcoding in the past?

A: In principle, the technology is also available to analyze this by mass spectrometry; however, the difficulty there lies in the fact that we don't ourselves have access to such a machine and don't have the expertise. We are forced to send it out. Even though we have the National Proteomics Center on our campus, it's still difficult to plug our samples in. It is costly and oftentimes central labs don't consider your sample urgent.

Q: What is your long-term vision for the Platinum instrument in your research?

A: Our lab is really dedicated to nanomedicine. So not just for CAR T cells, but you can also think of using protein barcoding for other applications where we want to target other tissues, and always the difficulty lies in the number of choices that you have. There's so much to vary in a nanoparticle, and the moment you step away from lipid nanoparticles and go to polymeric nanoparticles, you encounter the same things – the ratio between the different parts of the polymer. There's an endless number of choices, and you want to explore as many of them in one go as you can. Protein barcoding will also be pivotal for optimizing other aspects of CAR T-cell development. For example, by experimenting with various spacers between the recognizing fragment and the intracellular signal transfer part, we can enhance signal transduction or generate new signals. Testing these configurations in parallel allows for identifying the most effective designs.