

Schlussbericht zum DZIF-Vorhaben
Zur Veröffentlichung über die Technische
Informationsbibliothek - Deutsche Forschungsberichte
Hannover (TIB)

Vorabennummer: DZG 01.202
Vorhabentitel: Cell type specific targeting for future *in vivo*
delivery in cell & gene therapy
Förderkennzeichen: 8039201202 (UK FR)
8026201202 (LMU K)
8009201202 (UKE)

Verbundvorhaben

Beteiligte Einrichtung/en sowie Vorhabenlaufzeit/en

Projektkoordination	Laufzeitbeginn	Laufzeitende
Universitätsklinikum Freiburg	01.07.2024	- 31.12.2024

Verbundpartner	Laufzeitbeginn	Laufzeitende
Klinikum der Universität München	01.01.2023	31.12.2023
Universitätsklinikum Hamburg-Eppendorf	01.01.2023	31.12.2024

Kapitel 1: Kurzbericht

a) Ursprüngliche Aufgabenstellung und wissenschaftlicher/technischer Stand, an den angeknüpft wurde

Overall project objective: Join forces to improve current gene transfer techniques (Klinikum der Universität München (UK LMU), Universitätsklinikum Freiburg (UK FR) & Universitätsklinikum Hamburg-Eppendorf (UKE))

Ex vivo technologies for genetic modification of cells and tissues are labor-intensive, time-consuming, and correspondingly expensive. The underlying thesis of this project states that cell type-specific targeting will improve *ex vivo* procedures and particularly promote the development of *in vivo* applications. Such improvements will expand the availability of novel therapies based on genetic modifications.

Our cooperative project therefore pursued the goal of developing efficient targeting vectors for the targeted gene modification of defined cells or tissues, in order to promote broader application of *ex vivo* and *in vivo* gene therapies in the medium term.

b) Ablauf des Vorhabens

Klinikum der Universität München (UK LMU)

Work package 1 Virus-like particles (VLP) Development: Early involvement of the PEI was sought in order to appropriately consider feasibility of clinical translation and safety aspects. UKE and UK LMU have set-up a collaborative structure with the Paul-Ehrlich Institute by preparing documentation and Pre-Scientific Advice meeting in 2023. Upcoming meetings with PEI will be organized by UKE together with UK FR.

Work package 2 Testing of the vector systems for T cell engineering: Engineering of T cells was investigated by UK LMU in order to develop targeted delivery of synthetic gene products *ex vivo* and *in vivo*. Feasibility of CRISPR/Cas9-mediated genome editing *via* these targeted delivery systems was investigated. Primary T cells were genetically engineered using a variety of cargos in viral approaches. Therefore, GMP-compatible CRISPR/Cas engineering was developed in primary human T cells by UK LMU using GMP-grade reagents as well as electroporation devices. After transfer of the project to UK FR, a proof of concept of a non-viral approach, LNP targeting in T cells will be investigated.

Work package 3 on macrophages: Participation in WP 3 was transferred from UK LMU to UK FR.

Universitätsklinikum Freiburg (UK FR)

Work package 1 Virus-like particles (VLP) Development:

UK FR participated in Pre-Scientific Advice meeting with the Paul-Ehrlich Institute in 2024.

Work package 2 Testing of the vector systems for T cell engineering:

In WP2 we investigated non-viral targeting of primary human T cells by using an LNP approach. From a variety of possible cargos to be delivered by the LNPs, we decided on

mRNA, due to the advantage of its transient expression. In house production of control and CAR mRNA proved to work and led to protein expression in targeted cells.

GMP-compatible CRISPR/Cas engineering was developed in primary human T cells by UK LMU before, using GMP-compatible reagents as well as electroporation devices. No major contributions by UK FR were required from UK LMU to fulfil the tasks related to viral approaches.

Work package 3 on macrophages:

The here described project was part of the DZG Innovation Fund and included partners from DZGs outside of DZIF. Unlike what was planned in the project description, the targeting of macrophages with LNPs was not addressed by the DZIF partners. UK FR focused LNP targeting and feasibility experiments on primary T cells.

Universitätsklinikum Hamburg-Eppendorf (UKE)

UKE participated in WP1 and WP2

In WP1, we were able to produce and comprehensively analyze different VLPs based on both γ -retroviral and lentiviral vectors, as planned. The results have already been published (Wichmann et al 2023). In particular, we were able to show that a specific type of VLPs (so-called enhanced γ -retroviral VLPs) is best suited for the transfer of the CRISPR/Cas system.

As planned in WP2, we tested the suitability of the new VLPs developed in WP1 for the modification of primary human T cells in direct comparison with (i) mRNA electroporation, (ii) RNP electroporation, and (iii) transfection using lipid nanoparticles (LNPs) also produced in the laboratory. For the production of LNPs, the "Nanoparticle formulation system" Spark from Precision Nanosystems was acquired using our own funds. In direct comparison, it was shown that the LNPs were significantly superior to VLPs in the modification of primary T cells (unpublished data). Both mRNA and RNP electroporation are also efficient, but only suitable as *ex vivo* procedures. In contrast, VLPs and LNPs can potentially also be used *in vivo*. Since the production of VLPs is significantly more complex, especially regarding future GMP manufacturing, it can be concluded that LNPs have greater potential for the delivery of CRISPR/Cas components into T cells.

c) Wesentliche Ergebnisse und ggf. Zusammenarbeit mit anderen Forschungseinrichtungen

Klinikum der Universität München (UK LMU)

In WP1, UK LMU -together with UKE- has involved the national authorities Paul-Ehrlich Institute (PEI) in order to appropriately consider feasibility of clinical translation and safety aspects. A collaborative structure with PEI has been set-up and resulted in a first Pre-Advice Meeting at PEI in 2023. The next meeting with PEI is scheduled already.

For WP2, UK LMU has developed several strategies and platforms on engineering of T cells. Feasibility of CRISPR/Cas9-mediated genome editing in terms of single and double knock-outs as well as knock-ins with single and double knock-outs in an all-in-one approach have been set-up. Therefore primary T cells were genetically engineered using a variety of cargos in viral as well as non-viral approaches.

GMP-compatible CRISPR/Cas engineering was developed in primary human T cells using the DZIF supported GMP-electroporation device GTx (MaxCyte).

For scientific exchange regular meetings of the DZG Innovation Fund have been coordinated and moderated by UK LMU. Exchange of protocols has been coordinated by UK LMU.

Universitätsklinikum Freiburg (UK FR)

The main result was proof of concept for targeting primary T cells with LNPs and delivering functional mRNA, generated by in vitro transcription internally.

Regular exchanges regarding the protocols used and the results obtained took place with the project partners via web meetings

Universitätsklinikum Hamburg-Eppendorf (UKE)

The main result was the clear superiority of LNPs for transferring gene editing components into primary T cells.

Regular exchanges regarding the protocols used and the results obtained took place with the project partners via web meetings.

Kapitel 2: Eingehende Darstellung

a) Ausführliche Darstellung der erzielten Ergebnisse im Einzelnen

Klinikum der Universität München (UK LMU)

Work package 1 Virus-like particles (VLP) Development: As planned for WP1, UK LMU - together with UKE- has involved the national authorities Paul-Ehrlich Institute (PEI) in order to appropriately consider feasibility of clinical translation and safety aspects. A collaborative structure with PEI has been set-up and resulted in a first Pre-Advice Meeting at PEI in 2023. The next meeting with PEI is scheduled for Q4 2024 and will be coordinated by UKE together with UK FR.

Work package 2 Testing of the vector systems for T cell engineering: For WP2, UK LMU has developed several strategies and platforms on engineering of T cells. Feasibility of CRISPR/Cas9-mediated genome editing in terms of single and double knock-outs as well as knock-ins with single and double knock-outs in an all-in-one approach have been set-up. Therefore primary T cells were genetically engineered using a variety of cargos in viral as well as non-viral approaches.

GMP-compatible CRISPR/Cas engineering was developed in primary human T cells using the DZIF supported GMP-electroporation device GTx (MaxCyte).

Work package 3 on macrophages: Participation in WP 3 was transferred from UK LMU to UK FR.

For scientific exchange regular meetings of the DZG Innovation Fund have been coordinated and moderated by UK LMU. Exchange of protocols has been coordinated by UK LMU. From now on, responsibilities for the coordination and moderation of the meetings as well as the coordination of the exchange of protocols is taken over UK FR.

Universitätsklinikum Freiburg (UK FR)

Work package 2 Testing of the vector systems for T cell engineering: In WP2 we investigated non-viral targeting of primary human T cells by using an LNP approach. Different LNP compositions were assembled on a NanoAssemblr Spark System and evaluated towards their efficiency to lead to expression of mRNA in primary T cells. According to published results on a variety of cargos to be delivered by LNPs, we decided on mRNA as the type of cargo to follow up on. One advantage of mRNA is its transient expression. In house production of control and CAR mRNA by *in vitro* transcription proved to work and led to protein expression as detected in T cells following nucleofection. Targeting of primary human T cells with mRNA carrying LNPs led to expression of the respective proteins as well. Of note, incorporation of mRNA into LNPs and subsequent transduction of primary human T cells led to satisfying expression of control mRNA. However, expression efficiency of CAR protein after LNP targeting of T cells was not sufficient and needs further investigation.

GMP-compatible CRISPR/Cas engineering was developed in primary human T cells by UK LMU using GMP-compatible reagents as well as electroporation devices. No major contributions by UK FR were required from UK LMU to complete the tasks related to viral approaches.

Work package 3 on macrophages: The here described project was part of the DZG Innovation Fund and included partners from DZGs outside of DZIF. Unlike what was planned in the project description, the targeting of macrophages with LNPs was not addressed by the DZIF partners. UK FR focused LNP targeting and feasibility experiments on primary T cells.

Universitätsklinikum Hamburg-Eppendorf (UKE)

WP1: As planned, different retrovirus-based virus-like particles (RVLPs) were produced and tested. In particular, both γ -retrovirus and lentivirus-based VLPs were designed and directly compared for the first time. Based on our investigations, we were able to show that a specific type of VLPs (so-called enhanced γ -retroviral VLPs) is best suited for the transfer of the CRISPR/Cas system. The results obtained have already been published in Open access and are therefore widely available:

Wichmann M, Maire CL, Nuppenau N, Habiballa M, Uhde A, Kolbe K, Schröder T, Lamszus K, Fehse B*, Głów D* (2023) Deep Characterization and Comparison of Different Retrovirus-like Particles Preloaded with CRISPR/Cas9 RNPs. *International Journal of Molecular Sciences* **24**, 11399. (*equal contribution)

WP2 aimed to test the new vector systems developed in WP1 for the genetic *ex vivo* as well as the potential *in vivo* modification of T lymphocytes, especially regarding their suitability for the delivery of different cargos (RNA, proteins) for gene editing. The RVLPs were also to be directly compared with other vector systems already established in the laboratory as well as with the new lipid nanoparticles (LNPs).

As planned, the RVLPs were used to test CRISPR/Cas9-based gene editing systems in T cell lines as well as primary T cells. It was found that the efficiency in primary human T cells is significantly lower than that of LNPs or RNPs. In fact, with RVLPs in primary T cells, only low editing rates in the single-digit percentage range could be achieved, while these were an order of magnitude higher with VLPs and after RNP electroporation (unpublished data). Given these results, the unsuitability of RNPs for *in vivo* modification, and the significantly simpler production of LNPs, we have concluded to focus on LNPs in the future.

b) Meilensteine und Liefergegenstände

Klinikum der Universität München (UK LMU)

Meilensteine

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
2	Set-up of collaborative structure for T-cell specific targeting for future <i>in vivo</i> delivery	1	UK LMU, UKE, UK FR	31.12.2024		Nicht erreichbar	Milestone 2 ist für UK LMU nicht erreichbar. Wurde an UK FR übergeben.

Liefergegenstände

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
2	Set-up of GMP-compatible CRISPR engineering	2	UK LMU	31.12.2023		Erfüllt	
3	Proof of concept LNP targeting in T cells	2	UKE, UK LMU, UK FR	31.12.2024		Nicht erreichbar	Deliverable 3 ist für UK LMU nicht erreichbar. Wurde an UK FR übergeben.
4	LNP-based delivery strategies with feasibility experiments	3	UK LMU, UK FR	31.12.2024		Nicht erreichbar	Deliverable 4 ist für UK LMU nicht erreichbar. Wurde an UK FR übergeben.

Universitätsklinikum Freiburg (UK FR)

Meilensteine

Nr.	Titel	Arbeitspaket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
2	Set-up of collaborative structure for T-cell specific targeting for future <i>in vivo</i> delivery	1	UK LMU, UKE, UK FR	31.12.2024		Erfüllt	

Liefergegenstände

Nr.	Titel	Arbeit s-paket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
3	Proof of concept LNP targeting in T cells	2	UKE, UK LMU, UK FR	31.12.2024		Erfüllt	
4	LNP-based delivery strategies with feasibility experiments	3	UK LMU, UK FR	31.12.2024		Erfüllt	

Universitätsklinikum Hamburg-Eppendorf (UKE)

Meilensteine

Nr.	Titel	Arbeit s-paket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
1	Production and optimization of non-viral and viral vectors	1	UKE	31.12.2023		Erfüllt	
2	Set-up of collaborative structure for T-cell specific targeting for future <i>in vivo</i> delivery	1	UK LMU, UKE, UK FR	31.12.2024		Erfüllt	

Liefergegenstände

Nr.	Titel	Arbeit s-paket	Einrichtung	Datum lt. Vorhabenbeschreibung	Korrigiertes Datum	Status	Kommentar/Begründung
1	Development of retrovirus-based virus-like particles (VLPs)	1	UKE	31.12.2023		Erfüllt	
3	Proof of concept LNP targeting in T cells	2	UKE, UK LMU, UK FR	31.12.2024		Erfüllt	

c) Wichtigste Positionen des zahlenmäßigen Nachweises

Personalmittel

Klinikum der Universität München (UK LMU)

Einsatz: *in kind*

Sachverhalt: Exchange of protocols, preparation and moderation of Pre-Advice meeting with PEI, Set-up of GMP-compatible CRISPR engineering

Universitätsklinikum Hamburg-Eppendorf (UKE)

Einsatz: 93.722,41 €

Sachverhalt: Scientist (Postdoc) ca. 0,4 position as planned

Universitätsklinikum Freiburg (UK FR)

Einsatz: 79.865,30€

Sachverhalt: Scientist (Postdoc) 2x 6 months; Resources were spent according to plan.

Sachmittel (auch Aufträge)

Klinikum der Universität München (UK LMU)

Einsatz: *in kind*

Sachverhalt: Set-up of GMP-compatible CRISPR engineering

Universitätsklinikum Hamburg-Eppendorf (UKE)

Einsatz: 17.743,62 €

Sachverhalt: Plasmid preparation, mRNA generation, flow cytometry, molecular biology

Universitätsklinikum Freiburg (UK FR)

Einsatz: 28.451,75€

Sachverhalt: LNP targeting of primary human T cells

Investitionsmittel

Klinikum der Universität München (UK LMU)

Not applicable

Universitätsklinikum Hamburg-Eppendorf (UKE)

Not applicable

Universitätsklinikum Freiburg (UK FR)

Not applicable

Reisekosten

Klinikum der Universität München (UK LMU)

Not applicable

Universitätsklinikum Hamburg-Eppendorf (UKE)

Not applicable

Universitätsklinikum Freiburg (UK FR)

Not applicable

d) Notwendigkeit und Angemessenheit der geleisteten Arbeiten

Klinikum der Universität München (UK LMU)

The utilization of the resources was appropriate and necessary to achieve the results.

Universitätsklinikum Hamburg-Eppendorf (UKE)

The utilization of the resources was appropriate and necessary to achieve the results.

Universitätsklinikum Freiburg (UK FR)

The utilization of the resources was appropriate and necessary to achieve the results.

e) Voraussichtlicher Nutzen des Vorhabens, insb. die Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Klinikum der Universität München (UK LMU)

The work and achievements of UK LMU are necessary to successfully complete the project together with UKE and UK FR. UK FR requires the results of UK LMU to address further questions within the project.

Universitätsklinikum Hamburg-Eppendorf (UKE)

Building on the results of this project, the further development of LNPs (Lipid Nanoparticles) for efficient transfection of T cells both *in vitro* and *in vivo* has now become a central component of several (collaborative) projects of the applicants.

Universitätsklinikum Freiburg (UK FR)

Building on the results of this project, the further development of LNPs (Lipid Nanoparticles) for efficient transfection of T cells both *in vitro* and *in vivo* will become a central component of future (collaborative) projects of the applicants.

- f) Während der Durchführung des Vorhabens dem Zuwendungsempfänger bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen

Klinikum der Universität München (UK LMU)

The project may be performed as planned.

Universitätsklinikum Hamburg-Eppendorf (UKE)

Successful development of LNPs for T cell transfer has also been described by other groups.

Universitätsklinikum Freiburg (UK FR)

Successful development of LNPs for T cell transfer has been described by other groups.

- g) Erfolgte oder geplante Veröffentlichung der Ergebnisse nach Nr. 5 der Nebenbestimmungen für Zuwendungen (NABF/NKBF 2017)

Klinikum der Universität München (UK LMU)

No publication yet. Future publication in open, peer-reviewed Journal.

Universitätsklinikum Hamburg-Eppendorf (UKE)

Wichmann M, Maire CL, Nuppenau N, Habiballa M, Uhde A, Kolbe K, Schröder T, Lamszus K, Fehse B*, Głów D* (2023) Deep Characterization and Comparison of Different Retrovirus-like Particles Preloaded with CRISPR/Cas9 RNPs. *International Journal of Molecular Sciences* **24**, 11399. (*equal contribution)

Universitätsklinikum Freiburg (UK FR)

No publication yet. Future publication in open, peer-reviewed journal.