Symposium der Paul-Martini-Stiftung 2024

RNA-basierte Therapien: Neue Wirkstoffklasse, neue Wirkprinzipien

29.-30. November 2024 in Berlin

ABSTRACT-SAMMLUNG

Freitag, 29. November 2024

10:30 – 10:45 Uhr - Prof. Dr. Nikolaus Rajewsky

Max-Delbrück-Centrum für Molekulare Medizin (MDC)

Berliner Institut für Medizinische Systembiologie

RNA im erkrankten Gewebe – spatial omics und Indentifizierung potenzieller drug targets

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10:55 – 11:10 Uhr - Prof. Dr. Thomas Carell

Ludwig-Maximilians-Universität München

Chair for Organic Chemistry I

Department for Chemistry, Institute for Chemical Epigenetics

Chemie von RNA-Therapeutika

Nukleinsäuren etablieren sich derzeit als neuartige Medikamente und Vaccine. Sie erlauben es mit hoher Präzision an zelluläre Nukleinsäuren zu binden, um *splicing*-Prozesse zu beeinflussen oder mRNAs gezielt zu degradieren. Als Vakzine bringen sie den Code für virale Proteine in Zellen, was eine Immunantwort und damit Immunität gegenüber dem kodierten Protein und dem damit verbundenen Pathogen aufbaut.

Eines der Hauptprobleme bei der Entwicklung von Nukleinsäure-Therapeutika ist das effiziente *delivery* der Nukleinsäure in die Zellen hinein, verbunden mit einem *targeting* der Nukleinsäuren in spezifische Zellen. Derzeit erfolgt das *delivery* durch ein Verpacken der Nukleinsäuren z.B. in Liposomen. Das *targeting* gelingt mit Hilfe chemischer Zuckermodifikationen z.B. in Leberzellen. In dem Vortrag werde ich neue click-chemische Methoden vorstellen, mit denen Nukleinsäuren gezielt chemisch modifiziert werden können.[1] Neuartige Linker werden gezeigt, mit denen komplexe Zuckerstrukturen an die Nukleinsäuren effizient in Eintopf-Reaktionen angebracht werden können.[2] Die Verknüpfung findet in allen Fällen statt über Nukleobasen statt, die in Nukleinsäuren eingebaut werden, welche über eine zusätzliche Alkingruppe verfügen. Die chemischen Modifizierungseinheiten verfügen über Azid-Gruppen. Die bioorthogonale Reaktion der

Azide mit den Alkinen gelingt mit Hilfe der Cu-katalysierten Clickreaktion.[3] In dem Vortrag zeige ich ferner, dass der Ersatz der Uridin-Bausteine in mRNA durch alkin-tragende Nukleinsäuren die Stabilität der mRNA maßgeblich erhöht und es somit zu einer verlängerten Expression des kodierten Proteins kommt. In der Kombination des Austausches der Uridine durch alkin-substituierte Uridine und der click-chemischen Modifizierung der Nukleinsäuren mit komplexen Zuckerstrukturen in Eintopfreaktionen glauben wir chemische Methoden entwickelt zu haben, mit denen die Herstellung neuartiger RNA Therapeutika und Vaccine gelingen könnte.

[1] F. R. Traube, M. Stern, A. J. Tölke, M. Rudelius, E. Mejías-Pérez, N. Raddaoui, B. M. Kümmerer, C. Douat, F. Streshnev, M. Albanese, P. R. Wratil, Y. V. Gärtner, M. Nainytė, G. Giorgio, S. Michalakis, S. Schneider, H. Streeck, M. Müller, O. T. Keppler, T. Carell *Angew. Chem. Int. Ed.* **2022**, 10.1002/anie.202204556

[2] A.J. Tölke, J.F. Gaisbauer, Y.V. Gärtner, B. Steigenberger, A. Holovan, F. Streshnev, S. Schneider, M. Müller, T. Carell *Angew. Chem. Int. Ed.* **2024**, 10.1002/anie.202405161

[2] E.S. Schönegger, A. Crisp, M. Radukic, J. Burmester, T. Frischmuth, T. Carell *ChemBioChem* **2023**, 10.1002/cbic.202300701

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11:20 – 11:35 Uhr - Dr. Sebastian Motsch

BioSpring GmbH

Project Lead GMP and Large Scale Production

Upscaling im Bereich GMP-RNA-Herstellung (Abstract)

Oligonucleotides have rapidly developed to become one of the most sought-after classes of drugs in the world with an explosive growth of clinical development programs and about two new oligonucleotide drug products approved each year since 2016. Approved therapies were initially limited to the treatment of rare disorders, but the recent approval of lnclisiran for patients with atherosclerotic cardiovascular disease, one of the leading causes of mortality worldwide, marks a turning point in the field and oligo drugs have become drugs with the potential to treat diseases afflicting millions of people. For more than 40 years, solid-phase synthesis based on the phosphoramidite method has been the upstream process of choice, which presents several challenges in terms of sustainability, such as an unfavorable Process Mass lntensity (PMI) and the usage of considerable volumes of acetonitrile (ACN). With increasing batch sizes and production volumes, it is essential to move towards more sustainable oligonucleotide manufacturing processes to reduce the environmental footprint. Moreover, due to the limited scalability manufacturing processes must be fast and efficient to be able to run multiple batches in a short time frame to achieve very large production volumes by combining batches.

The characteristics of solid-phase synthesis (e.g. high equivalents of chemicals, multiple washing steps) and the usage of chromatographic purification system during downstream-processing contributes to the unfavorable PMI of solid-phase based oligonucleotide API manufacturing processes. This talk will present a PMI-driven optimization of current manufacturing processes: For a commercial ASO manufacturing process it is estimated that 1 /3 of raw materials of can be spared through PMI-driven

process optimization, mainly by minimizing the amount of acetonitrile in washing steps, the detritylation volumes and the amount of HPLC purification buffers.

As solvents contribute significantly to the hazardousness of oligonucleotide synthesis and especially acetonitrile is neither considered "benign" in terms of safety, health and environment, nor available through biorenewable sources much focus is on replacing ACN, especially as washing solvent. This talk will present a case study on the usage of alternative solvents in a current synthesis protocol (synthesis of 24-nt long, fully thiolated DNA strand "CpG 7909", an adjuvant in vaccines): By incorporating additional solvents (Candidate 1: Ethyl Acetate, Candidate 2: "Solvent 6") in selected washing steps, significant amounts of the acetonitrile could be replaced with solvents with improved sustainability characteristics. The produced crude material was comparable to the reference material in terms of yield, purity and impurity profile.

As solid-phase synthesis processes are limited in scalability and only capable of producing Kg batch sizes, very large production volumes can be only achieved by combining multiple batches. Therefore, processes need to be efficient and fast to allow a very fast turnaround. This talk will demonstrate how manufacturing process and facility may be set-up for manufacturing > 1 metric ton oligonucleotide per year.

Finally, an outlook about alternative synthesis approaches with the potential to be capable for manufacturing of multiple hundreds of Kg oligonucleotide per batch and a low environmental footprint will be presented: Alternative approaches are based on solution-phase synthesis (chemically/enzymatically) due to the higher scalability. However, the feasibility of alternative approaches to supply materials of sufficient purity and amount for clinical trials still needs to be established.

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12:05 – 12:20 Uhr - Prof. Dr. Dr. Stefan Engelhardt

Technische Universität München

TUM School of Medicine and Health

Institut für Pharmakologie und Toxikologie

Antisense-RNA gegen Mikro-RNA bei fibrotischen Erkrankungen

Die konventionelle Pharmakotherapie stößt bei einer Reihe von Indikationen zunehmend an ihre Grenzen. Insbesondere bei fibrotischen Erkrankungen stehen nur wenige geeignete Wirkstoffe zur Verfügung, so dass ein dringender Bedarf an neuen therapeutischen Ansatzpunkten besteht. MicroRNAs sind als zentrale Regulatoren der Genexpression an verschiedenen pathologischen Prozessen beteiligt, unter anderem an der Entstehung von Organfibrosen. Unsere Arbeiten haben mehrere MicroRNAs in Makrophagen identifiziert, deren Funktion entscheidend zur Entstehung von entzündungsassoziierten Myokard- und Lungenfibrosen beiträgt. Eine große Herausforderung bei der Entwicklung nukleinsäurebasierter Therapien ist die oft geringe Aufnahmeeffizienz in die Zielzellen. Wir konnten kürzlich zeigen, dass durch die Kopplung von Oligonukleotiden an komplexe Zuckermoleküle eine hocheffiziente und selektive Aufnahme in Makrophagen erreicht werden kann. Der verwendete Trimannose-Ligand bindet an Mannose-Rezeptoren, die spezifisch auf Makrophagen exprimiert werden, und ermöglicht so eine zelltypspezifische Hemmung fibroseinduzierender RNAs. Dieses neue

Therapieprinzip hat inzwischen alle Phasen der präklinischen Entwicklung erfolgreich durchlaufen und steht nun kurz vor der klinischen Erprobung am Menschen.

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12:30 – 12:45 Uhr - Prof. Dr. Dr. med. Thomas Thum

Medizinische Hochschule Hannover

Antisense-RNA gegen Mikro-RNA bei Herzerkrankungen

Antisense-RNA gegen Mikro-RNAs könnte sich als eine vielversprechende Strategie zur Behandlung von Herzerkrankungen erwiesen. Die Forschung auf diesem Gebiet begann mit der Entdeckung, dass Mikro-RNAs (miRNAs) als regulatorische Elemente im Genom eine Schlüsselrolle bei der Steuerung vieler physiologischer und pathologischer Prozesse spielen, einschließlich der Herzfunktion und -gesundheit. Zahlreiche Studien haben gezeigt, dass bestimmte miRNAs bei Herzinsuffizienz, Myokardinfarkt und anderen kardiovaskulären Erkrankungen überaktiv sind und die Entwicklung und das Fortschreiten der Krankheit fördern. Durch die gezielte Blockierung dieser krankheitsfördernden miRNAs mittels Antisense-Technologie können potenziell neue Therapieansätze entwickelt werden.

Die Antisense-RNA-Technologie nutzt kurze, synthetische RNA-Stränge, die spezifisch an krankheitsrelevante miRNAs binden und deren Aktivität neutralisieren. Dies ermöglicht es, die dysregulierte Genexpression zu korrigieren und damit die zugrunde liegenden Krankheitsprozesse positiv zu beeinflussen. In den letzten Jahren haben vorklinische Studien den therapeutischen Nutzen dieser Strategie in Tiermodellen für verschiedene Herzerkrankungen gezeigt, einschließlich einer verbesserten Herzfunktion und Reduktion von Gewebeschäden nach Herzinfarkten.

Darauf aufbauend wurden erste klinische Studien initiiert, die die Sicherheit und Wirksamkeit von Antisense-RNA gegen miRNAs bei Patienten mit Herz-Kreislauf-Erkrankungen evaluieren. Eine der weltweit größten klinischen Studien zu einem mikroRNA-basierten Therapeutikum (HF-REVERT) testet derzeit Effekte bei Patienten nach Myokardinfarkt. Diese innovativen therapeutischen Ansätze könnten langfristig zu einem neuen Ansatz in der Behandlung von Herz-Kreislauf-Erkrankungen führen.

Zusammengefasst bieten Antisense-RNA-Therapeutika gegen mikroRNA eine neuartige und gezielte Methode zur Modulation von Krankheitsprozessen im Herzen, aber auch in vielen anderen Organsystemen.

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12:55 – 13:10 Uhr - Prof. Dr. Matthis Synofzik

Hertie-Institut für Klinische Hirnforschung

Abteilung Neurologie mit Schwerpunkt neurodegenerative Erkrankungen

Development of a platform for individual patient-customized RNA antisense therapies

Background: the medical and societal need. Rare neurological diseases (RND) – while each individually rare – together pose a major societal health burden, comprising of

>2.000 severely debilitating or life-threatening diseases. This devastating individual and societal burden contrasts with a striking lack of treatments, with 95% of RNDs lacking disease-modifying treatment options. The stark absence of treatments – and even of promising treatment developments – is in part due to the rarity of individual RNDs, their sheer number, and large mutational heterogeneity. Yet it is in particular driven by the absence of appropriate translational drug development methodology, with both current drug development frameworks and current trial methodology falling short for the plethora of ultra-rare diseases where only very few or single patients exist worldwide.

Our approach: To overcome these challenges, we are establishing a scalable platform approach for development of patient-customized programmable antisense oligonucleotide (ASO) therapies for individuals with severe RNDs. This innovative "out-of-the-box" drug development platform allows scalability despite the rarity of the targeted diseases and mutations by leveraging a widely shared disease-overarching interventional principle: ASO-mediated splice modulation. This unifying biological principle allows targeting individuals' personal ultra-rare or even private mutation; yet at the same time allows scalability across hundreds, if not thousands of individuals and diseases, by leveraging the multiple methodological and translational synergies in ASO development across an – in principle endless – number of ASOs and mutations for severe RNDs (Fig 1).

Fig.1 **Platform for development of patient-customized programmable ASO therapies**: tailored to each individual's mutation, yet scalable to multiple patients and diseases alike by streamlining all of its development modules.

Current state of our drug development platform. Our platform approach includes all components of a preclinical to first-in-human drug development pipeline: from omics-based theranostics, preclinical target validation to innovative trial methodology and preparation of EMA-advised personalized ASO trials. Importantly, as a platform approach, it allows streamlining of all these modules, resulting in a highly effective, sustainable and scalable platform for patient-customized programmable ASO therapies. Our platform currently comprises preclinical ASO development for >40 different mutations/individuals, representing >10 different RNDs. Just for one severe pioneering showcase RND – Ataxia teleangiectasia (A-T) –, we have developed ASO strategies for 10 different mutations. The pharmacological module of our platform is thereby streamlined by all developed ASOs utilizing the same 2`MOE-PS ASO backbone as the already widely studied, regulatory approved and post-approval clinically monitored ASO Nusinersen (for spinal muscular atrophy). This paradigm allows leveraging the large body of corresponding pharmacological data, regulatory blueprints, and clinical experience, enabling rapid transition to first-in-human trials.

Overall impact. Our scalable platform approach for development of patient-customized ASO therapies sets the stage for preclinical, clinical and regulatory patient-customized brain precision therapies. It has already proven successful from preclinical design to

clinical translation, with first patient-customized ASO first-in-human treatments already ongoing in clinics for Ataxia teleangiectasia.

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14:20 – 14:35 Uhr - Prof. Dr. rer. nat. Jörg Vogel

Julius-Maximilians-Universität Würzburg

Institut für Molekulare Infektionsbiologie

Helmholtz Institute for RNA-based Infection Research (HIRI)

Helmholtz Centre for Infection Research (HZI)

Antiinfektive RNA-Therapie

Neue programmierbare ANTIBIOTIKA auf Grundlage von RNA

Antisense technologies have the potential to form a foundation for the development of a new generation of antibiotics. Upon delivery into the bacterial cell, short antisense oligonucleotides (ASOs) or mimics thereof can directly modulate bacterial gene expression, for example, by suppressing mRNA translation of an essential target protein. The programmable nature of ASOs, which is based on simple base-pairing rules, allows rational and specific drug design and opens myriad applications including the rapid development of ASOs that can kill emerging pathogens, sensitize drug-resistant strains, or block expression of key virulence factors all while sparing the native microbiome. However, despite ample proof-of-concept for efficacy against a diverse range of bacterial pathogens *in vitro* and *in vivo*, ASOs are yet to advance to the point of drug approval. This talk will also discuss potential new applications of such "asobiotics" that go beyond the mere killing of pathogens, for example, interfering with undesired activities of commensals and even targeting the natural enemies of microbes, that is, phages. There now is considerable momentum to advance ASOs from a promising area of research to an applied technology capable of addressing the growing antimicrobial resistance crisis and providing solutions for the analysis of genetically intractable microbes.

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14:45 – 15:00 Uhr - Prof. Dr. Thomas Pietschmann

TwinCore - Zentrum für Experimentelle und Klinische Infektionsforschung GmbH

Leiter Institut für Experimentelle Virologie

RNA-basierte antivirale Vakzine

Impfungen gehören zu den erfolgreichsten medizinischen Interventionen überhaupt. Ein Erreger wie das Pockenvirus, das allein im 20. Jahrhundert über 300 Millionen Menschenleben gefordert hat, konnte durch Impfungen nicht nur kontrolliert, sondern eliminiert werden. Impfstoffe haben die Krankheitslast von Infektionen wie Polio, Masern und Diphtherie so weit reduziert, dass diese Infektionen – zumindest in Europa – fast aus unserer Wahrnehmung verschwunden sind. In jüngster Zeit hat der Einsatz von Impfstoffen, insbesondere von mRNA-basierten Impfstoffen, die Coronavirus-Pandemie beendet. Mehr als fünf Milliarden Menschen wurden mit mRNA-Impfstoffen behandelt und profitierten vom Schutz dieser neuen Impftechnologie. Aufbauend auf langjähriger RNA-

Forschung, bahnbrechenden Erkenntnissen über die Mechanismen, die die Expression und Immunantwort auf mRNA-basierte Antigene steuern, sowie Durchbrüchen im Bereich der mRNA-Formulierung im Vorfeld, kombiniert mit beschleunigten Zulassungsverfahren, wurde der Weg für die Verfügbarkeit wirksamer mRNA-Impfstoffe in weniger als einem Jahr nach Ausbruch der Pandemie geebnet.

So eröffnen sich heute völlig neue Chancen, Infektionskrankheiten mit Hilfe innovativer mRNA-basierter Impfstoffe weiter zurückzudrängen. Dabei mahnt die WHO, nicht nur das Marktpotenzial, sondern auch den medizinischen und gesellschaftlichen Bedarf als Maßstab für die Priorisierung der Impfstoffentwicklung heranzuziehen. Unter den Top 4-Kandidaten für die Impfstoffentwicklung der WHO befinden sich mit dem Hepatitis C-Virus (HCV) und dem Humanen Immundefizienz-Virus HIV-1 zwei virale Erreger, die weltweit verbreitet sind und trotz verfügbarer antiviraler Wirkstoffe jährlich fast eine Million Menschenleben fordern. Da beide Erreger in nahezu allen (HIV) bzw. den meisten (HCV) Fällen eine chronische Infektion auslösen, also besondere Eigenschaften entwickelt haben, um das Immunsystem zu überlisten, kommt der Impfstoffforschung gegen diese beiden Erreger nicht nur eine besondere Bedeutung zu, sondern sie steht auch vor besonderen Herausforderungen. Im Falle von HCV scheint die Hürde für die Entwicklung einer Schutzimpfung deutlich niedriger zu sein, da sich das Virus im Gegensatz zu HIV nicht unwiderruflich in das Erbgut unserer Körperzellen einschneiden kann. Darüber hinaus sind bis zu 30% der HCV-Exponierten in der Lage, eine schützende Immunantwort zu entwickeln, die das Virus innerhalb weniger Wochen kontrolliert und eliminiert. Diese schützende Immunität wird im Wesentlichen durch Antikörper gegen hochkonservierte Bereiche der HCV-Hüllproteine vermittelt. HCV besitzt jedoch besondere Eigenschaften, die die Bildung solcher schützender Antikörper oder deren Wirkung einschränken.

Im Rahmen des Vortrags werden aktuelle Entwicklungen auf dem Gebiet der mRNA-Impfstoffforschung gegen Infektionserreger kurz zusammengefasst. Anschließend werden Chancen und Grenzen der mRNA-Impfstofftechnologie am Beispiel von HCV und im Vergleich zu anderen klassischen Impfverfahren diskutiert. Abschließend wird ein Ausblick gegeben, wie die interdisziplinäre mRNA-, Pathogen- und Immunforschung zusammen mit den Computerwissenschaften in Zukunft weitere Potentiale für den Einsatz von mRNA-Vakzinen zur Kontrolle und Eliminierung von Infektionen erschließen könnte.

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15:30 – 16:45 Uhr - Prof. Dr. med. Veit Hornung

Ludwig-Maximilians-Universität München

Genzentrum

Self or non-self? Detection of nucleic acids in the endolysosome

A central function of our innate immune system is to detect microbial pathogens by the presence of their nucleic acid genomes or their transcriptional or replicative activity. In mammals, a receptor-based system – represented by pattern recognition receptors (PRRs) – is primarily responsible for the detection of "non-self" nucleic acids. In recent years, tremendous progress has been made in identifying the key sensing and signaling components required for this complex task. The first group of PRRs identified as nucleic acid sensing receptors are the toll-like receptors (TLRs). TLRs are expressed as transmembrane receptors with their ligand binding domain facing either the extracellular space or the luminal compartment. A distinct evolutionary subset of TLRs is located in the

endolysosomal compartment, which in the human system includes TLR7, TLR8 and TLR9. While TLR9 recognizes single-stranded DNA with unmethylated CG motifs, which are indeed suppressed in the host genome, TLR7 and TLR8 have evolved to recognize RNA degradation products. Although there has been considerable research on RNA-sensing TLRs, our understanding of their capability to differentiate between non-self and self-RNA remains limited, particularly considering the prevalence of self-RNA in the endolysosomal compartment. In this talk, I will provide an update on our recent work on this topic and present some novel insights into how TLR7 and TLR8 discriminate self from non-self.

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16:45 – 17:00 Uhr - Prof. Dr. Özlem Türeci

BioNTech SE

Medizinische Geschäftsführerin

RNA-basierte anti-Tumor-Vakzine

k. A. – virtueller Vortrag

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17:10 – 17:25 Uhr - Prof. Dr. Olivia Merkel

Ludwig-Maximilians-Universität München

Department Pharmazie

Optimization or organ and cell specific RNA delivery

Pulmonary delivery of RNA has been widely successful for treating viral infections and other respiratory disorders [1]. However, although direct delivery to the lungs is possible, efficient intracellular delivery of RNA in the lungs at low toxicity is still a major challenge because of extra- and intracellular barriers the lung as an organ poses [2]. Whereas administration of "naked" RNA requires large doses to account for inefficient transport through mucus and cellular internalization of these negatively charged macromolecules, viral vectors can cause severe immunological responses and are mostly not specific for a certain target cell population. Targeted non-viral RNA carriers, on the other hand, offer several advantages [3]. Considering that cationic lipid-based RNA nanocarriers are easily destabilized by phospholipid-containing airway surface liquid (surfactant) in the lower airways [4], RNA formulation with biodegradable polymer-based nanocarriers will be presented. Cationic polymers can also interact nonspecifically with respiratory mucus in the upper airways and can thus get entrapped resulting in limited diffusion [4]. Therefore, we have developed shielded polymer nanocarriers that are decorated with poly(ethylene glycol) (PEG) [5-8], oligo(ethylene glycol) [9] or the glycoprotein transferrin [10] on their surface to decrease non-specific interactions with mucus for unhampered diffusion through mucus [8]. Additionally, we have modified RNA nanocarriers with targeting ligands (Figure 1) to be taken up by specific cell types in the lung [10] or in tumor models [11-13]. This approach has been described for successful T cell targeting [10, 14] and was recently exploited for delivery to angiotensin-converting enzyme 2 (ACE2) expressing cells. (ACE2) has been established as a functional host receptor on pulmonary epithelium for both SARS-CoV-1 and SARS-CoV-2 [15-17]. To deliver RNA preferentially to epithelial cells that express ACE2, we attached a short

protein sequence that is part of the conserved region of the receptor binding domain (RBD) of the virus' spike protein [18] or an anti-ACE2 antibody to the latest generation of poly(beta aminoester) (PBAE)-based polyspermines [19, 20] (Figure 1).

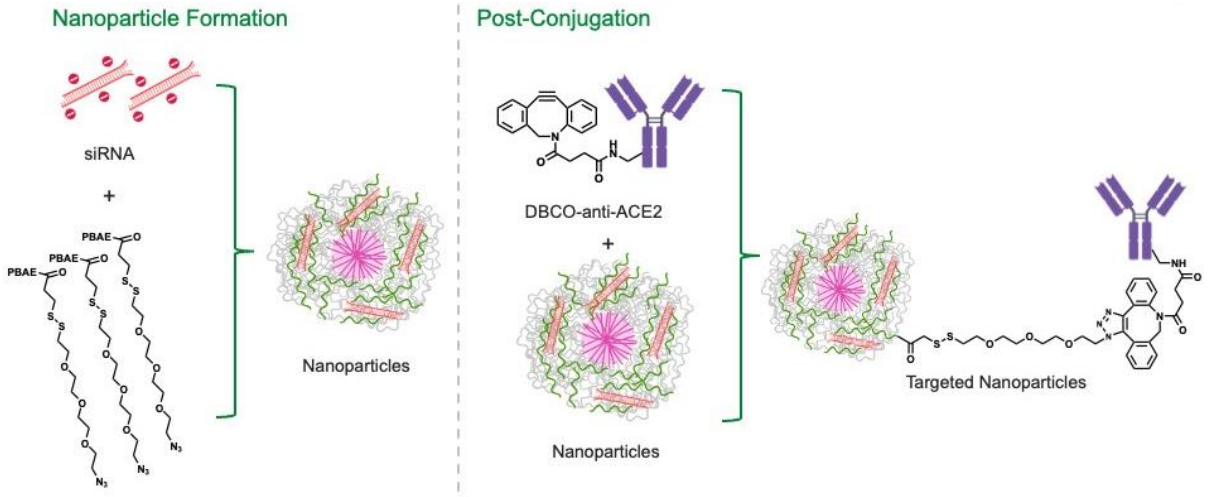

Figure 1: Nanoparticle formulation with PBAE-based polyspermine polymers with azide modification and siRNA as surrogate. DBCO-modified antibodies are clicked onto the nanoparticles post particle formation for surface modification.

Coupling via click chemistry was previously established in our group [21, 22]. The nanocarriers were tested with regards to stability against shear force and temperature increase during nebulization and were spray dried to obtain inhalable powders [19] with improved storage conditions [23]. Cell type tropism and specificity were investigated in vivo [24].

[1] O.M. Merkel, I. Rubinstein, T. Kissel, siRNA Delivery to the lung: What's new?, Advanced Drug Delivery Reviews 75 (2014) 112-128.

[2] O.M. Merkel, T. Kissel, Nonviral Pulmonary Delivery of siRNA, Acc Chem Res 45(7) (2012) 961-70.

[3] K.A. Whitehead, R. Langer, D.G. Anderson, Knocking down barriers: advances in siRNA delivery, Nat Rev Drug Discov 8(2) (2009) 129-38.

[4] N. Sanders, C. Rudolph, K. Braeckmans, S.C. De Smedt, J. Demeester, Extracellular barriers in respiratory gene therapy, Adv Drug Deliv Rev 61(2) (2009) 115-27.

[5] O. Merkel, A. Beyerle, D. Librizzi, A. Pfestroff, T. Behr, B. Sproat, P. Barth, T. Kissel, Nonviral siRNA Delivery to the Lung: Investigation of PEG-PEI Polyplexes and Their In Vivo Performance, Molecular Pharmaceutics 6(4) (2009) 1246-1260.

[6] D.P. Feldmann, Y. Xie, S.K. Jones, D. Yu, A. Moszczynska, O.M. Merkel, The impact of microfluidic mixing of triblock micelleplexes on in vitro / in vivo gene silencing and intracellular trafficking, Nanotechnology 28(22) (2017) 224001.

[7] Q. Zhong, O.M. Merkel, J.J. Reineke, S.R. da Rocha, Effect of the Route of Administration and PEGylation of Poly(amidoamine) Dendrimers on Their Systemic and Lung Cellular Biodistribution, Mol Pharm 13(6) (2016) 1866-78.

[8] I. d'Angelo, G. Conte, G. Costabile, D. Baldassi, F. Quaglia, R. Sorrentino, O.M. Merkel, F. Ungaro, Enabling pulmonary delivery of siRNA in cystic fibrosis lung inflammation: therapeutic potential of hybrid lipid/polymer nanoparticles, Journal of Aerosol Medicine and Pulmonary Drug Delivery 32(3) (2019) A36-A26.

[9] D.P. Feldmann, Y. Cheng, R. Kandil, Y. Xie, M. Mohammadi, H. Harz, A. Sharma, D.J. Peeler, A. Moszczynska, H. Leonhardt, S.H. Pun, O.M. Merkel, In vitro and in vivo delivery of siRNA via VIPER polymer system to lung cells, J Control Release 276 (2018) 50-58.

[10] Y. Xie, N.H. Kim, V. Nadithe, D. Schalk, A. Thakur, A. Kilic, L.G. Lum, D.J. Bassett, O.M. Merkel, Targeted delivery of siRNA to activated T cells via transferrin-polyethylenimine (Tf-PEI) as a potential therapy of asthma, J Control Release 229 (2016) 120-9.

[11] D.P. Feldmann, S. Jones, K. Douglas, A.F. Shields, O.M. Merkel, Microfluidic Assembly of siRNA-Loaded Micelleplexes for Tumor Targeting in an Orthotopic Model of Ovarian Cancer, Methods Mol Biol 1974 (2019) 355-369.

[12] S.K. Jones, K. Douglas, A.F. Shields, O.M. Merkel, Correlating quantitative tumor accumulation and gene knockdown using SPECT/CT and bioluminescence imaging within an orthotopic ovarian cancer model, Biomaterials 178 (2018) 183-192.

[13] L. Liu, M. Zheng, D. Librizzi, T. Renette, O.M. Merkel, T. Kissel, Efficient and Tumor Targeted siRNA Delivery by Polyethylenimine-graft-polycaprolactone-block-poly(ethylene glycol)-folate (PEI-PCL-PEG-Fol), Mol Pharm 13(1) (2016) 134-43.

[14] D.C. Jurgens, J.T. Muller, A. Nguyen, O.M. Merkel, Tailoring lipid nanoparticles for T-cell targeting in allergic asthma: Insights into efficacy and specificity, Eur J Pharm Biopharm 198 (2024) 114242.

[15] J. Lan, J. Ge, J. Yu, S. Shan, H. Zhou, S. Fan, Q. Zhang, X. Shi, Q. Wang, L. Zhang, X. Wang, Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature 581(7807) (2020) 215-220.

[16] M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G. Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, S. Pohlmann, SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor, Cell 181(2) (2020) 271-280 e8.

[17] A. Demogines, M. Farzan, S.L. Sawyer, Evidence for ACE2-utilizing coronaviruses (CoVs) related to severe acute respiratory syndrome CoV in bats, J Virol 86(11) (2012) 6350-3.

[18] S. Wang, F. Guo, K. Liu, H. Wang, S. Rao, P. Yang, C. Jiang, Endocytosis of the receptor-binding domain of SARS-CoV spike protein together with virus receptor ACE2, Virus Res 136(1-2) (2008) 8-15.

[19] Y. Jin, X. Wang, F. Adams, T.M.H. Ngo, O.M. Merkel, Synthesis and application of spermine-based amphiphilic poly(β-amino ester)s for siRNA delivery, Biomacromolecules (2024).

[20] A.P.E. Kromer, F. Sieber-Schafer, J. Farfan Benito, O.M. Merkel, Design of Experiments Grants Mechanistic Insights into the Synthesis of Spermine-Containing PBAE Copolymers, ACS Appl Mater Interfaces 16(29) (2024) 37545-37554.

[21] S.K. Jones, V. Lizzio, O.M. Merkel, Folate Receptor Targeted Delivery of siRNA and Paclitaxel to Ovarian Cancer Cells via Folate Conjugated Triblock Copolymer to Overcome TLR4 Driven Chemotherapy Resistance, Biomacromolecules 17(1) (2016) 76-87.

[22] B. Gabold, F. Adams, S. Brameyer, K. Jung, C.L. Ried, T. Merdan, O.M. Merkel, Transferrin-modified chitosan nanoparticles for targeted nose-to-brain delivery of proteins, Drug Deliv Transl Res 13(3) (2023) 822-838.

[23] C.M. Zimmermann, L. Dessloch, D.C. Jurgens, P. Luciani, O.M. Merkel, Evaluation of the effects of storage conditions on spray-dried siRNA-LNPs before and after subsequent drying, Eur J Pharm Biopharm (2023).

[24] F. Adams, C.M. Zimmermann, D. Baldassi, T.M. Pehl, P. Weingarten, I. Kachel, M. Kranzlein, D.C. Jurgens, P. Braubach, I. Alexopoulos, M. Wygrecka, O.M. Merkel, Pulmonary siRNA Delivery with Sophisticated Amphiphilic Poly(Spermine Acrylamides) for the Treatment of Lung Fibrosis, Small 20(22) (2024) e2308775.

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Samstag, 30. November 2024

09:00 – 09:15 Uhr - Dr. Katharina Höfer

Max-Planck-Institut für terrestrische Mikrobiologie

SYNMIKRO

RNAylation of proteins: Discovery of molecular glue to link RNA and proteins

„Leben beruht auf Beziehungen zwischen Molekülen", schrieb der berühmte Biologe Linus Pauling. Wechselwirkungen zwischen Proteinen und RNA bestimmen beispielsweise die Translation, die Reparatur der Erbinformation oder den Transport zellulärer Bausteine. Dabei handelt es sich um vorübergehende Kontakte zwischen RNA und RNA-bindenden Proteinen, die auf bestimmten RNA-Strukturen oder -Sequenzen beruhen.
Wir entdeckten kürzlich, dass Protein und RNA auch fest, in einer sogenannten kovalenten Bindung, miteinander verknüpft sein können. Die feste Verknüpfung von RNA und Proteinen, die als „RNAylierung" bezeichnet wird, stellt ein neuartiges Konzept der natürlichen RNA-Protein-Interaktion dar. Die RNAylierung wurde erstmals in Bakterien beobachtet, die von Bakteriophagen infiziert sind. Dabei konnte gezeigt werden, dass ein Enzym des Bakteriophagen spezifisch die Ribosomen des Bakteriums, die für die Proteinbiosynthese verantwortlich sind, RNAyliert. Diese kovalente Modifikation der Ribosomen könnte die bakterielle Translation hemmen und es dem Phagen ermöglichen, die Synthese seiner eigenen Proteine zu steuern. Dies versetzt den Phagen in die Lage, die Kontrolle über das Bakterium zu übernehmen, neue Phagen zu produzieren und diese schließlich durch die Lyse des Bakteriums freizusetzen.
Zudem eröffnet die Entdeckung der RNAylierung ganz neue Wege in der Forschung. Zum Beispiel könnte die RNAylierung zukünftig ein Werkzeug für die Synthetische Biologie und Medizin werden. Als „molekularer Klebstoff" ließe sie sich zur Bildung spezifischer RNA-Protein-Konjugate einsetzen, um die Eigenschaften von Proteinen und Nukleinsäuren kombiniert, beispielsweise als mRNA-Therapeutika, nutzen zu können.

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09:25 – 09:40 Uhr - Dr. Steffen Thirstrup

CMO, European Medicines Agency EMA

Regulatory challenges and opportunities – how best to engage with European regulatory authorities (focus RNA-based therapies)

This presentation will briefly give an overview of currently approved therapies based on RNA technology and the Agency's activities on this topic in relation to scientific advice to developers.

I will give an overview of the possible pathways to engage in discussion with regulatory authorities in EU, both nationally and directly at EMA. These include scientific advice including qualification of new biomarker technologies, engagement with the EU Innovation Network and the EMA Innovation Task Force, as well as dedicated contact points and support for small-medium sized (SME) companies and academia. My presentation can be shared with the audience and will include active hyperlinks to the relevant information and contact points at EMA for further information and support.

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09:50 – 10:05 Uhr - Dr. Stefan Blesse (hält Vortrag von Dr. Ulrich Granzer)

Granzer Regulatory Consulting & Services GmbH

Chief Scientific Officer

Challenges and (potential) solutions in the development and registration process of RNA based new drugs

Fomivirsen was approved by the FDA in 1998 and by the EMA in 1999 as the very first antisense drug used to treat CMV retinitis in patients with AIDS. Following this first generation antisense molecule a couple of more approvals have happened, like the one for Patisiran, a double-stranded siRNA that causes degradation of mutant and wild-type TTR mRNA through RNA interference and, probably as the most widely used drug ever in a single year, Comirnaty, a LNP stabilized mRNA vaccine "for active immunization to prevent COVID-19 caused by SARS-CoV-2, in individuals 12 years of age and older" as it is described in the Summary of Product Characteristics (SmPC) issued by the EMA.

The above examples, to name only a few, show that meanwhile pharma companies and regulators have found ways to develop and approve rather different types of RNA based drugs.

They are used for very rare diseases like CMV retinitis, SMA type 1, but also for large indications like Inclisiran (Leqvio), which is "indicated in adults with primary hypercholesterolemia (heterozygous familial and non-familial) or mixed dyslipidemia, as an adjunct to diet" (SmPC), and, obviously, Comirnaty.

While manufacture and routine analytics necessary for a successful drug development and registration were difficult in the beginning, they are now following clear sets of rules, which experts in the field can handle.

Preclinical development, in particular toxicology, was dominated by investigating non-human primates but the situation is changing rathe rapidly towards other animals

including pigs and dogs as non-rodents as well as mice and rats. A clear development pattern necessary for a later drug approval becomes visible and, hence, a clear pathway to approval can be defined in discussions between regulatory experts in (biotech)industry and regulators in authorities.

The remaining and most expensive part of development is the least defined, clinical.

In the orphan space 11 patients sufficed for a first approval, in the vaccine area it was 45,000, to name the span and bandwidth.

Two main items remain as the biggest development and regulatory challenges: dosing and dosing intervals.

Whereas dosing seems to be for most of these new drugs in the area of 0.5 – 5 (-10) mg/kg bodyweight, the dosing interval lies between a few weeks and several months and it is completely uncorrelated to blood pK, as the substances are usually only active after internalization into cells, thus leaving the plasma and becoming broadly undetectable by standard pK measurements.

The presentation will highlight the items and issues above and will also discuss ways towards registration.

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10:45 – 11:00 Uhr - Dr. Joel G. Rurik

Karolinska Institutet

Center for Infectious Medicine, Department of Medicine Huddinge

Targeting Pathogenic Fibroblasts with RNA-Engineered T Cells

Cardiac disease afflicts millions of people globally and is hallmarked by substantial alterations to constituent cells. Fibroblasts are mesenchymal cells of particular interest within the diseased heart because they contribute to pathogenic fibrosis (stiffening of the tissue) and serve as pro-inflammatory signaling hubs. Following injury fibroblasts proliferate and activate, fundamentally changing their molecular signature. We leverage the unique molecular signature of pathogenic fibroblasts (namely fibroblast activation protein or FAP) as a target for redirected cytotoxic lymphocytes. Chimeric antigen receptor (CAR) T cells are typically manufactured outside the body using lenti-/retroviral vectors or CRISPR/Cas9 to permanently integrate the CAR sequence into the T cell genome. While advantageous for anti-cancer therapeutic applications, a permanent population of anti-fibrotic CAR T cells in circulation poses significant risk to subsequent acute injuries such as myocardial infarction or bodily injuries where fibrotic, scar-forming responses are critical for maintaining tissue integrity. Therefore, we turned to the power of therapeutic mRNA for transient FAP CAR expression. mRNA was synthesized with 1-methylpseudouridine and purified to avoid activating innate immune sensors. The mRNA was packaged within lipid nanoparticles (tLNP) which were then decorated with anti-CD5 antibodies (a receptor normally expressed by T cells) to mediate T cell uptake and subsequent CAR expression. In proof-of-concept experiments, we show that this novel approach generates anti-fibrotic CAR T cells entirely within the body. These transiently engineered T effector cells eliminate the pathogenic activated fibroblasts and improve the heart’s function in a chronic mouse model of pressure-overload injury. Perhaps most excitingly, the molecular phenotype of activated fibroblasts is consistent across other

fibrotic diseases in non-cardiac tissues. Therefore, we tested the mRNA/tLNP technology to generate FAP CAR T cells in numerous mouse models of fibrotic diseases including rheumatoid arthritis, liver and kidney diseases, with promising initial results. These exciting proof-of-concept experiments demonstrate how a uniform, mass-manufacturable drug (mRNA in tLNPs) is capable of transiently generating potent cell therapies entirely within the body. This technology eliminates the expensive, bespoke manufacturing of CAR T cells, while also enabling repeat dosing. All together, we propose a novel, cost-effective and accessible approach to combat fibrotic diseases.