European Biotechnology

Spring 2025

Oligonucleotides and Peptides

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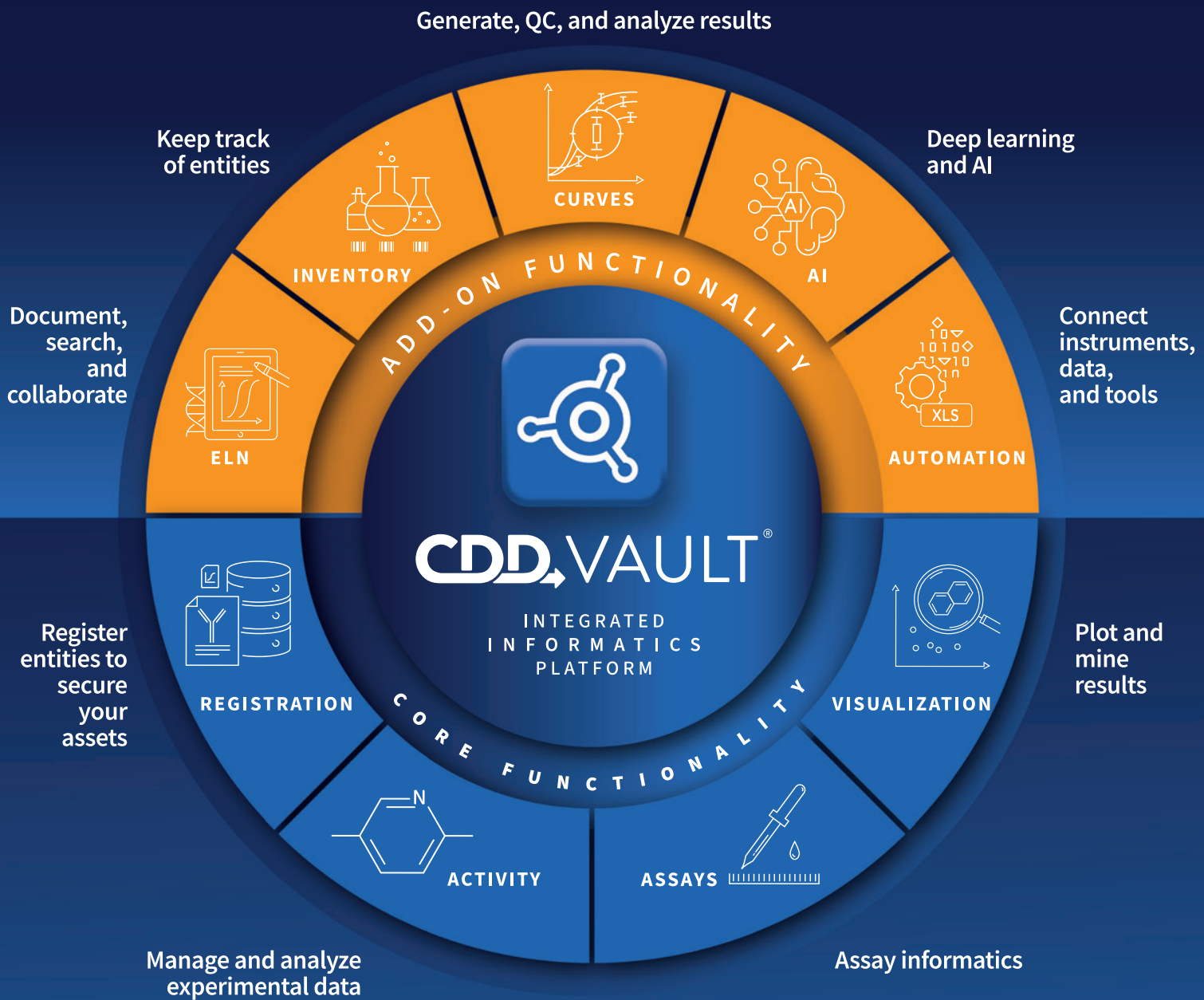

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Megatrend oligo-based and peptide drugs

BIOMANUFACTURING Peptide drugs such as the glucagon-like peptide-1 (GLP-1) receptor agonists semaglutide and tirzepatide alone recorded global sales of US$25.3bn in 2024, US$9.62bn of which in Europe. The market for gene and cell therapy contract manufacturing, oligonucleotide synthesis (US$9.1bn) and the production of viral vectors and plasmids (US$3.3bn), which is currently growing at an annual rate of 27%, is also expanding considerably. But there is still plenty of room for technological improvement.

Production capacities for GLP-1 receptor agonists are currently barely able to keep up with demand. That's reason enough for the manufacturers of appetite suppressants, diabetes drugs and potential drugs for currently 27 co-morbidities to scale up their production on a large scale, as there are hardly any manufacturing problems. Rather, the Big 20 are struggling to divide up the market with ever new combination approaches to make the peptide drugs more effective (see European Biotechnology 3/2024).

However, while these first generation of blockbusters reduces muscle mass as well as fat and must be taken for a lifetime to avoid a rebound effect, new approaches that are still in the early stages of development promise lasting resistance to weight gain by not targeting appetite, but instead transforming energy-storing white fat cells, which interfere with the hormone balance, into energy-consuming brown fat cells. One exam-

	HSV1	AVV	LV	AdV
Carrying capacity	> 30kb	< 4kb	8kb	~ 10kb
In vivo dosing[1]	Yes	Yes	No	Yes
No baseline antibody exclusion criteria[2]	Yes	No	Yes *(ex vivo)*	No
Repeat (flexible) dosing[3]	Yes	No	N/A	No
Tissue long-term expression	Yes	Yes	Yes	No
No integration into host cell DNA	Yes	Yes	No	Yes

Fig. 1: Comparison of the properties of common gene therapy vectors with the HSV-1 vectors of the French start-up EG 427 SA

Picture: © EG 427

ple is Resalis Therapeutics srl, a spin-off from the Italian gene therapy forge San Raffale Institute for Gene Therapy (see interview on p. 42).

Oligos instead of peptides

At the beginning of the year, following a €10m Series A financing round, it was able to spring a surprise: even before the start of clinical trials of its LNA antisense oligonucleotide (LNA-Aso) RES-10, the French pharmaceutical giant Sanofi SA secured the option for one of the world's first gene therapies in a blockbuster indication with an equity investment. RES-10 targets the inhibition of the regulatory microRNA-22, which converts white fat cells into brown fat cells and thus counteracts obesity.

Another example of an antisense nucleotide with blockbuster potential is the aso inhibitor CDR132L of the regulatory miRNA-132, clinically developed by the German company Cardior Pharmaceuticals, which reversed cardiac hypertrophy, the fibrotic thickening of the heart wall that causes heart failure, in a Phase II trial. Cardior was acquired by Novo Nordisk last year.

Recent figures from IQVIA suggest that antisense oligonucleotides (ASOs) make up 28% of the cardiometabolic RNA drug pipeline including disorders such as diabetes, obesity and its cardiovascular/neurological co-morbidities.

As RNA drugs such as Asos, siRNAs or CRISPR-based gene therapies can modulate any target encoded in the human genome, while AI can automatically exclude unsuitable hits, the corresponding market and pipelines are currently growing very rapidly, especially in Asia. In comparison, proteins as classical targets make up only 1.5% of the human genome, and at most 14% of them have binding sites that are accessible to biologics and small molecules.

Vector-based gene therapies

In addition to the 18 approved, scalable and multi-administered oligonucleotide-based gene therapies to date, which are usually delivered to the target site encapsulated in lipid nanoparticles (LNPs) or other nanocarriers, vector-based single-use gene therapies are also attracting a great deal of attention from investors. All gene therapies approved to date are still authorised for the treatment of rare diseases. Both, gene therapies and CAR-T or TCR-T cell therapies, which use viral vectors constructed from plamids to equip immune cells with synthetic recombinant receptors (CARs), suffer from high production costs. These are partly due to the low degree of automation in production, laborious engineering but especially because they work with autologous cells. Biomanufacturing is the cost driver here. Due to the high costs involved, healthcare systems are extremely reluctant to reimburse the costs, if at all.

Vector optimisation

Nevertheless, Big Pharma continues to invest heavily in the production of viral vectors (see Fig. 1), which transport the synthetic gene constructs ex vivo into target cells (CAR/TCR-T and NK cells) or in vivo to the target cells.

Developers are increasingly focussing on indications with blockbuster potential. Roche AG, which has invested €90m in a production facility for adeno-associated virus (AAV) vectors at its German site in Penzberg, is targeting neurological diseases, for example. Nevertheless, the production of AAV vectors often still suffers from suboptimal efficiency, i.e. poor yields of functional virus capsids. In their recent US$1bn collaboration with Dyno Therapeutics, their AAV capsid platform is being used to optimise vectors for *in vivo* gene therapies against neurological diseases.

The Danish start-up Fuse Vectors A/S, which recently closed a US$5.2m pre-seed financing round to advance its proprietary enzymatic virus-free AV capsid assembly technology, is also committed to optimising the yield of functional AAV capsids (see interview, p 46).

Meanwhile, competitors such as French company EG 427 SA, which raised €27m in Series B funding in February, have developed HSV1 vectors with a loading capacity of more than 30kB as an alternative to

the existing lentiviral, adenoviral and AAV vectors (see Fig. 39)

The optimisation and automation of the production of AAV, adenoviral, lentiviral and other vectors, which deliver the gene sequences to the target site for expression, is currently on the upswing again, according to Dr Martin Schleef, Managing Director of Plasmid Factory GmbH in Bielefeld, Germany, which specialises in the production of plasmids and minicircle DNA.

Non-viral vectors

"The market is currently being driven primarily by the success of CAR-T cell therapies. However, these are expensive and harbour safety risks due to the use of lentiviral vectors, which preferentially integrate into active genes and can therefore induce cancer instead of curing it," says Schleef. "A cost-effective and scalable solution that has already been clinically validated is the patent-protected approach pursued by T-CurX of transfecting CAR gene constructs directly into the T cell in a virus-free manner using transposons" (doi: 10.3390/ijms252413685).

The cost savings are indeed immense, as the costly co-transfection with three to four plasmids to generate a lentivirus is no longer necessary. Instead, T-CurX utilises two so-called minicircle DNAs - one for transporting the target DNA into the cell and one that encodes the transposase. These show no toxicity compared to plasmids, says Schleef, and transfect T cells with comparable efficiency as viral vectors transduce them. In addition, the T-CurX Sleeping Beauty transposons only integrate statistically into genes, which is an advantage over lentiviruses. According to T-CurX founder Michael Hudecek, who want to democratise cancer research by providing cost-effective CAR-T similars to approved CAR-T cell therapies, transfection efficacy of minicircle DNA in CAR-T cell development is near 60%. According to Schleef, they can even be designed in such a way that they no longer do so. The international competition, which focuses on optimising AAV vector yield, is still sceptical. However, Schleef can see from the incoming orders that they are testing the minicircles: "Those who have tried it are fascinated," says Schleef, "we are in the final stages of qualifying and certifying our GMP system after ArchiMed 2022's investment." ■

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Minicircle DNA

Minicircle DNA are small circular plasmid derivatives without origin of replication and slection marker that have been freed from all prokaryotic vector parts. They have been applied as transgene carriers for the genetic modification of mammalian cells, with the advantage that, since they contain no bacterial DNA sequences and less CpG motifs, they are less immunogenic than plasmids. The smaller size of minicircles also extends their cloning capacity and facilitates their delivery into cells. Their preparation usually follows a two-step procedure: Production of a parental plasmid with eukaryotic inserts) in *E. coli*. Induction of a site-specific recombinase at the end of this process but still in bacteria followed by the excision of prokaryotic vector parts via two recombinase-target sequences at both ends of the insert and recovery of the resulting minicircles e.g. by affinity chromatography.

The purified minicircle can be transferred into the recipient cell by transfection or lipofection. The smaller size of minicircles compared with standard plasmid vectors makes for greater transfection and expression efficiency, while the gene of interest is more stable expressed. ■

Towards a LNA-ASO-based obesity gene therapy

OBESITY Just before going into Phase I testing, Italian Resalis Therapeutics srl has secured €10m in a Series A financing and an equity investment of Sanofi SA. European Biotechnology spoke with Alessandro Toniolo, CEO of Resalis, about the challenges to commercialise an obesity gene therapy targeting miRNA 22.

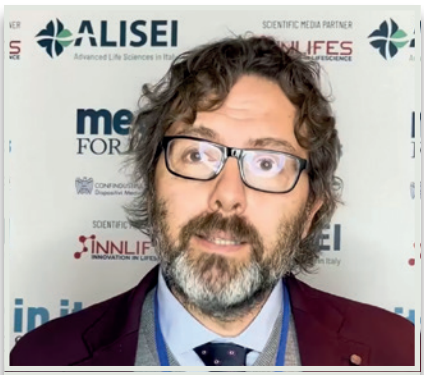
EuroBiotech_The results of inhibiting the regulatory microRNA 22(-3p) with a complementary LNA antisense nucleotide codenamed RES-10 in mouse models of obesity are impressive. Would you please briefly describe these and explain what processes in the human body miRNA-22 regulates?

Alessandro Toniolo_miR-22 plays a key role in lipid metabolism, mitochondrial function, and adipose tissue regulation. Preclinical studies conducted by our co-founder and Chief Scientific Officer, Riccardo Panella and his team, have shown that inhibiting miR-22 leads to significant metabolic improvements. This includes restored lipid biosynthesis, enhanced mitochondrial biogenesis, and the transformation of fat-storing white adipose tissue into energy-consuming brown adipose tissue. These combined effects reduce fat accumulation and increase energy expenditure, making weight reduction independent of food intake. RES-010 is designed to target miR-22, which could then modulate these pathways to support metabolic health. The promising preclinical findings support RES-010's ongoing clinical evaluation.

EuroBiotech_What are Resalis' preclinical toxicity, immunogenicity, and druggability data of RES-10?

Toniolo_Preclinical toxicology package studies demonstrated a favourable safety and tolerability profile supporting the further clinical development. Moreover, RES010 specifically showed no signs of triggering an immune response.

EuroBiotech_How is RES-10 transported to the site of action?



Alessandro Toniolo joined Resalis Therapeutics srl as CEO in October 2022 after 20 years in positions with increasing responsibility in the pharma industry. He worked in commercial roles at Italian, European and global companies including Merck, Pfizer, Shire and Novartis. Prior to joining Resalis, Alessandro was Novartis' Head of Immunology as well as Head of Respiratory and Allergy franchises in Italy. He is also a business angel supporting multiple oncology startup companies on behalf of Italian Angels for Growth.

Toniolo_RES-010 is formulated for systemic administration as a weekly subcutanous injection of the naked ASO. It is designed to efficiently reach its target tissues, ensuring selective engagement with its target.

EuroBiotech_What gap could Resalis' gene therapy fill in the treatment of obesity if RES-10 successfully completes clinical trials?

Toniolo_RES-010 is not a gene therapy as it does not alter gene sequences. It is an antisense oligonucleotide (ASO) that can modulate mRNA expression by targeting a microRNA, miR-22. MicroRNAs (miRNAs) are small non-coding RNAs that are approximately 21-25 nucleotides in length and function by regulating gene expression. They bind to messenger RNAs (mRNAs) and inhibit their translation into a protein, reducing the target protein's presence in cells. By inhibiting miR-22, we aim to restore the metabolic balance disrupted by obesity. RES-010's mechanism is distinct from current treatment options focusing on appetite suppression. Based on our preclinical results, we also believe that our candidate has the potential to be combined with treatments targeting GLP-1 RAs (GLP-1 receptor agonists) to provide more sustainable long-term metabolic improvements, filling a critical gap in current obesity treatment.

EuroBiotech_Resalis has received financial support from Sanofi surprisingly early after a Series A financing. How long will the money last and how is the risk distributed until the completion of Phase II development?

Toniolo_Our financial position allows us to advance RES-010 through key clinical milestones. We are focused on efficient resource allocation and leveraging strategic partnerships to support long-term development.

EuroBiotech_What competing approaches are you aware of, what are their strengths and weaknesses and how high do Resalis and Sanofi estimate the market potential for RES-10 on this basis?

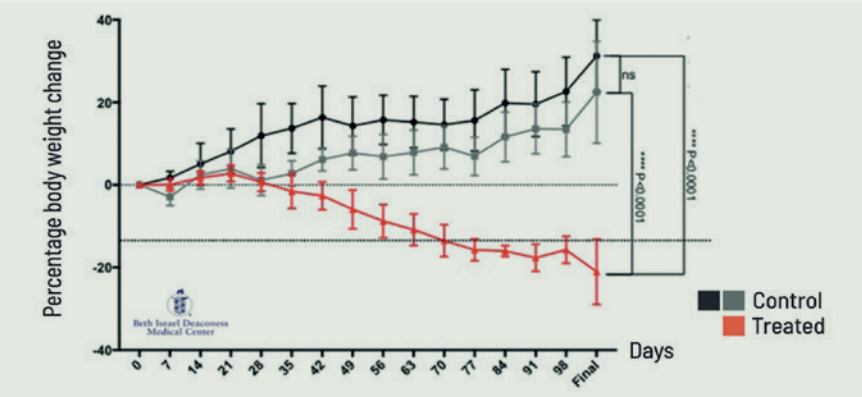
Pictures: Resalis Therapeutics srl/vimeo/innlifes.com

Toniolo_Most anti-obesity therapies do not directly target the underlying metabolic dysfunctions of obesity. RES-010 takes a different approach by modulating multiple interconnected metabolic pathways, positioning it within the new generation of multi-target therapies. As a miRNA-based intervention, it offers a novel and more comprehensive alternative to receptor-targeting drugs, with the potential to reshape obesity treatment by addressing its root metabolic causes.

EuroBiotech_How long will the follow-up period of your 48-patient Phase I safety and dose escalation study be, and when will you publish the first results?

Toniolo_The study is progressing as planned, and data will be disclosed in a timely manner, following rigorous analysis.

EuroBiotech_Is the study sufficiently powered to proceed directly to a pivotal study after first efficacy signals and an acceptable safety profile have been shown?



Administation of RES-010 led to a 20% reduction in fat after 100 days in mice models.

Toniolo_The Phase I trial is designed to assess safety, tolerability, and pharmacokinetics. We are confident that these data will provide a strong foundation for the next steps in clinical development, including a potential pivotal study.

EuroBiotech_What are the next relevant steps for Resalis?

Toniolo_We are focused on advancing the clinical development of RES-010 in obesity. In parallel, we are exploring additional indications where miR-22 modulation could offer therapeutic benefits. With RES-010's unique MoA, we are pursuing a multiple-indications strategy to expand its therapeutic potential. ■

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Picture: Resalis Therapeutics srl

Enhancing macromolecule drug discovery with CDD

DATA MANAGEMENT The landscape of macromolecule drug discovery is evolving rapidly, with oligonucleotides and peptides leading the way in innovative therapeutic applications. CDD Vault, a leading scientific data management system, significantly improves the way researchers manage macromolecule data by introducing a dedicated Macromolecule mode that seamlessly integrates with modern workflows.

› Mariana Vaschetto, PhD, Head of Operations EMEA, Collaborative Drug Discovery

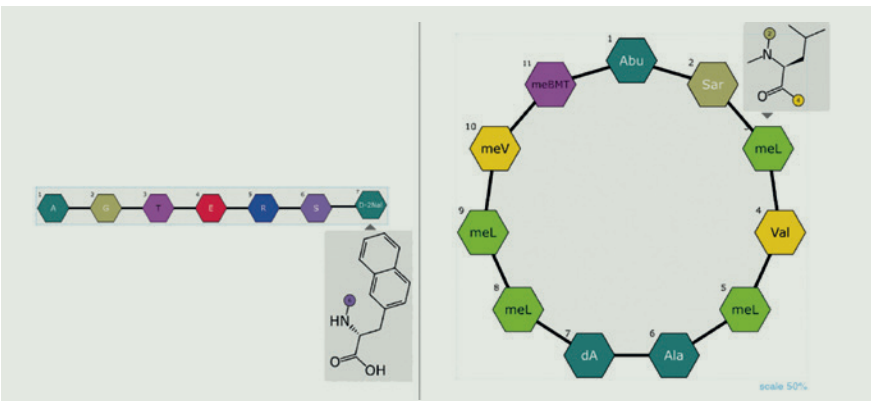
From a data perspective, macromolecules are difficult to manage due to their inherent complexity. To accelerate research, CDD Vault introduces a more efficient approach to registering macromolecules.

Simplified registration workflow

Researchers can now register individual molecules directly or upload bulk datasets using an easy-to-use interface. Whether working with linear or cyclic peptides, single-stranded RNA, or double-stranded DNA, sequences can be input using standard or custom codes. These sequences are automatically converted into structured molecular files, ensuring seamless integration with existing data. Metadata and experimental information are captured alongside molecular structures, making data management more comprehensive and efficient.

Intuitive structure editing

Building on CDD Vault's strong chemistry and biology foundation, the new Structure Editor Macromolecule Mode enhances how DNA, RNA, and peptide sequences are composed, edited, and visualised. Researchers can view sequences as letter codes or as molecular components, making it easier to analyse their structures. A built-in RNA builder allows for precise customisation of nucleotides, including sugar, base, and phosphate modifications. Users can also browse a growing library of unnatural amino acids and effortlessly upload or draw sequences using intuitive tools like Ketcher. Additionally, the ability to toggle between monomeric and full atomistic views provides greater flexibility in understanding complex macromolecular architectures.



Visualising structures of monomers from macromolecules registered in CDD Vault

Enhanced visualisation

Understanding macromolecular structures is critical for effective drug development. With CDD Vault, researchers can now explore molecular structures with greater interactivity. By simply hovering over a monomer, they can reveal detailed chemical structures and attachment points. The software intelligently orients molecular previews, making it easier to analyse modifications and interactions at a glance. This dynamic visualisation ensures that scientists, even those unfamiliar with CDD Vault, can quickly grasp key structural insights.

A commitment to innovation

This release is part of CDD Vault's ongoing commitment to improving the registration and analysis of modified bioconjugates. With continuous updates driven by expert user feedback, CDD Vault is poised to remain at the forefront of macromolecule drug discovery informatics. Researchers can now register, manage, and visualise complex biomolecular entities with ease, accelerating the path to groundbreaking therapeutics.

For further details, please visit *www.collaborativedrug.com*, or contact *info@collaborativedrug.com* ■

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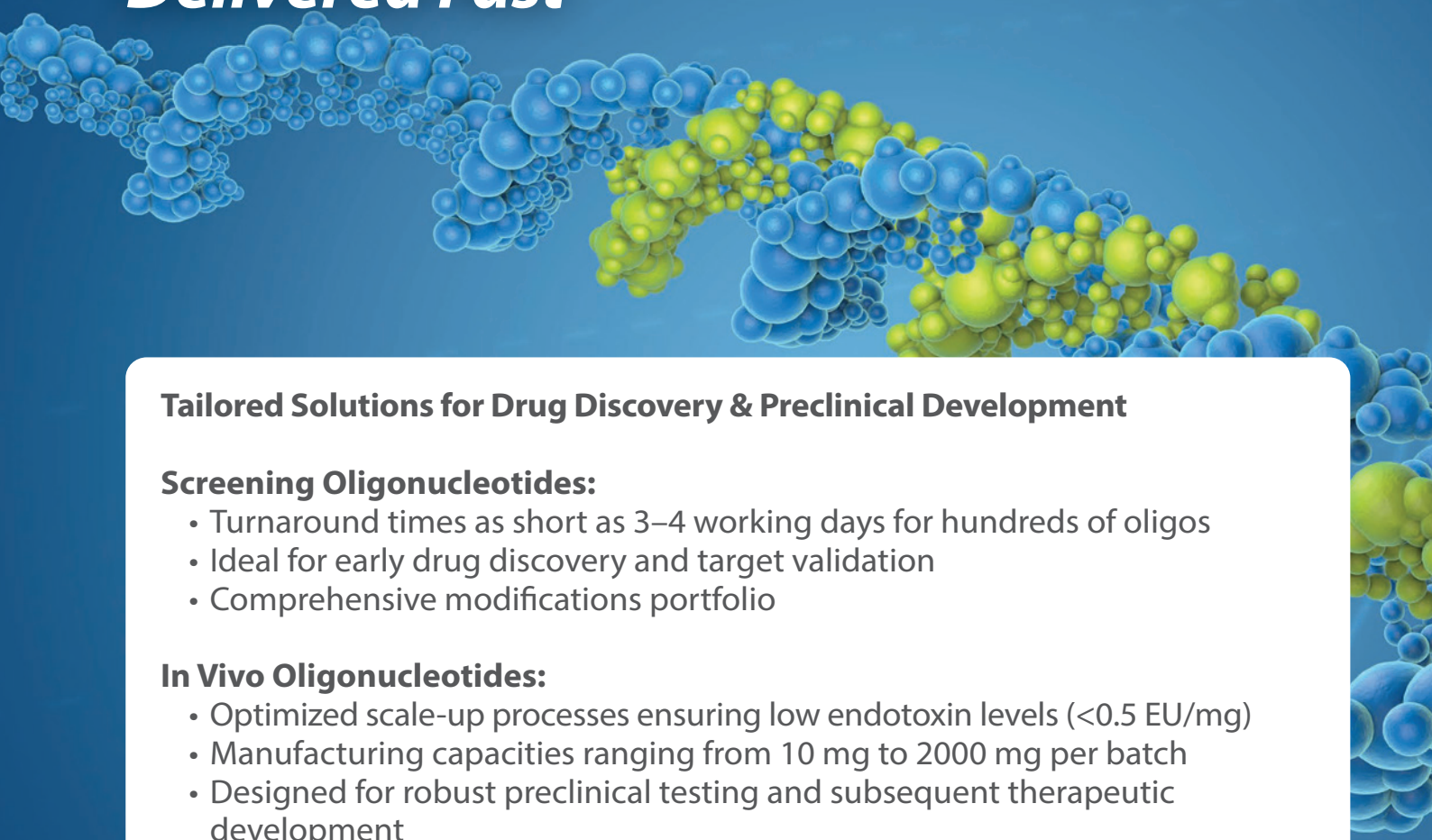

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Making AAV gene therapy faster and better

ADENO-ASSOCIATED VIRUS VECTORS AAVs Danish start-up Fuse Vectors ApS bagged pre-seed financing to advance its breakthrough cell-free technology for a faster, safer and more scalable gene therapy. European Biotechnology spoke with Jordan Turnbull, Co-CEO, CSO and co-founder of Fuse Vectors.

EuroBiotech_High production costs of gene therapies hinder their reimbursement and profitability. How does Fuse Vectors intend to change this?

Jordan Turnbull_For decades, gene therapy's potential has been limited by development processes first developed in the 1980s, which often result in inefficient products that drive up cost and increase toxicity risks in patients. Fuse Vectors is making gene therapy faster, better, and more affordable. Our novel process takes just hours per batch, significantly accelerating drug development, and ensures that more than 99% of the AAV capsids carry the target product, reducing unnecessary waste and inefficiencies that drive up costs. With the reduced time, expense, and risks associated with the novel process, we hope to incentivise more drug developers to enter the gene therapy space, expanding treatment options for diseases that currently have no cure.

EuroBiotech_Could you please describe exactly what differentiates your platform from other production methods for AAV vectors for gene therapies?

Turnbull_The key differentiator of our platform is the removal of the cell from the AAV gene therapy production equation. Because we work outside the cellular environment, we can cut development times from months to years to hours. Additionally, our system optimizes for >99% AAV capsids filling with the target gene to increase drug product quality, improve outcomes and decrease patient risks. Our technology is also modular and adaptable, meaning it can be used across different diseases and gene therapy programmes without the need for major re-engineering. We see the technology as more than just an innovation, but rather a quantum leap that will reshape the future of gene therapies.

Jordan Turnbull, Henrik Stage and Benjamin Blaha co-founded Fuse Vectors ApS in 2022. Jordan (right) serves as co-CEO and CSO, previously leading viral vector development teams in Boeh-ringer Ingelheim and Novo Nordisk. Henrik is Executive Chair, bringing huge experience in M&As and financing. Benjamin is serving as Co-CEO and CTO with huge process development expertise from his many years in the gene therapy industry.

EuroBiotech_... and how exactly it works, please?

Turnbull_Fuse Vectors' process breaks free from the constraints of cell biology. Thus, we can control and optimise the filling mechanism, the process by which the therapeutic gene is loaded into the AAV delivery vehicle, beyond what is feasible in living cells, allowing us to produce AAV gene therapy at the speed and quality not achievable with current technology. This also potentially allows us to fill novel capsid designs that were previously unmanufacturable resuscitating previously cancelled novel capsid projects. With this redefined AAV production process, Fuse Vectors is poised to become the universal solution for gene therapy needs.

EuroBiotech_Finally, what are the most important next steps to make your dual business plan work?

Turnbull_ Our first avenue involves collaborating with pharma and biotech companies as well as academic institutions. Simultaneously, we are committed to developing our own pipeline. While we are currently in the early stages of commercialisation and pre-revenue, we have proactively engaged in discussions with over a dozen partners set to trial our technology in the coming weeks, and we hope to convert these collaborations into revenue-generating relationships by the end of the year. The recent US$5.2m pre-seed funding, led by HCVC and supported by the BioInnovation Institute, EIFO, and Innovation Fund Denmark, is instrumental in accelerating the development of our Fuse Technology. This investment also enables us to mature current partnerships and advance our internal therapeutic pipeline. Looking ahead, we are actively seeking collaborations to develop new gene therapies, aiming to expand our impact and bring innovative treatments to patients worldwide. ■

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Pictures: Fuse Vectors A/S

Precision oligonucleotides for therapeutics

MANUFACTURING Therapeutic oligonucleotides are rapidly transforming drug development by enabling precise gene modulation strategies. Microsynth empowers biotech, pharmaceutical, and academic researchers with high-quality therapeutic oligonucleotides, supporting every stage from sequence design to preclinical development. With tailored solutions, advanced automation, and expert design support, we accelerate your therapeutic programmes with precision and speed.

› Elges Lardi, Business Development Therapeutic Oligonucleotides, Microsynth AG

Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) play key roles in gene modulation strategies. ASOs are single-stranded oligonucleotides that hybridise with target RNA sequences via complementary base pairing, inducing RNase H-mediated degradation or steric blocking of translation. In contrast, siRNAs are double-stranded molecules that integrate into the RNA-induced silencing complex (RISC) to degrade complementary mRNA sequences. Both approaches offer targeted gene silencing, making them promising tools for treating genetic disorders, viral infections, and oncological indications. The therapeutic potential of these novel long-lasting or even curative treatment approaches is underscored by the market approval of eleven ASOs and six siRNAs as drugs to date.

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- **Rapid turnaround times:** As short as three to four working days for screening oligos and five to six days for scale-up batches, accelerating timelines without compromising quality.

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