

Programmable cell therapies: Vlad Seitan

Combining gene editing and RNAi for advanced therapies

The emergence of RNA interference (RNAi) technologies in early 2000s came at a highly opportune time, coinciding with the publication of the first draft of the human genome, thus offering researchers a powerful toolkit to functionally annotate newly discovered genes at unprecedented scale. These technologies held tremendous promise – not only in research but also as a new class of therapeutics capable of silencing disease-causing genes with precision.

Since then, RNAi has been on a rollercoaster journey. The initial wave of enthusiasm was blunted by practical challenges – issues with delivery, off-target effects, and inconsistent silencing efficiency limited the clinical translation of RNAi-based therapies. The arrival of genome editing technologies further shifted attention away from RNAi, with the newer technologies dominating the field for much of the past decade, stealing the limelight and ultimately big pharma interest and investment.

Yet, RNAi is now experiencing a resurgence. As the field has matured, early limitations were addressed, and two decades after the initial discovery, the first small interfering RNA (siRNA) drug, Onpattro from Alnylam Pharmaceuticals Inc, was finally approved in 2018. This was followed by five more siRNA drugs receiving marketing approval in quick succession, bringing big pharma companies back into the RNAi arena, and sealing a comeback that seemed unlikely only a decade earlier. Today, multiple RNAi drugs are in development, and in a notable move, one of the original CRISPR gene editing companies – CRISPR Therapeutics AG – has recently announced a partnership with Sirirus Therapeutics to co-develop siRNA therapeutics. This partnership underscores that, for all the powerful applications of gene editing, RNAi approaches have unique advantages and are better suited for certain treatments.

Complementary technologies

At Laverock Therapeutics, we view gene editing and gene silencing not as competing technologies, but as complementary, and are seeking to lay a bridge between the genome editing revolution and the revival of RNAi. We think these technologies can expand each other's capabilities when combined, hence we have developed a next-generation gene silencing platform that draws on strengths of both approaches. The result is a new programmable gene silencing technology that combines the stability of gene editing with the versatility of RNAi. This platform enables the creation of advanced therapeutics uniquely suited to challenging diseases in oncology and beyond, particularly those where traditional gene knockout strategies fall short.

Laverock's gene silencing technology represents a novel, more physiological way of harnessing the RNAi pathway. Instead of introducing exogenous siRNA oligonucleotides or shRNA transgenes, this approach relies on making small, targeted edits to the cell's own microRNA (miRNA) genes, effectively hijacking endogenous miRNA loci to silence

desired target genes. The edits are only a few nucleotides long and are confined to the miRNA hairpin region, with no additional regulatory elements being introduced.

As a result, the edited locus produces a new silencing RNA (sRNA) without changing the locus's natural expression programme. In other words, an endogenous miRNA gene is turned into an "expression vector" for a therapeutic sRNA – the identity of the silencing RNA is changed, but the locus's normal expression programme is preserved. The platform is agnostic to the choice of editing tool used to introduce the miRNA gene edits, ensuring the platform can evolve alongside advances in gene editing technologies.

Our gene silencing platform is enabled by the extensive redundancy within the network of endogenous miRNAs. Many miRNA genes belong to wider families closely related in sequence that regulate the same physiological targets, and disrupting single miRNA genes is often phenotypically inconsequential, unless the entire family is targeted. The platform exploits this by repurposing non-essential miRNA genes, so that the edits do not disrupt normal cellular physiology.

Importantly, the platform overcomes several fundamental limitations of classical gene-silencing technologies, while also expanding gene editing's capabilities beyond simple knockouts or corrections to enable programmable gene silencing. It ultimately enables gene silencing that is inherently stable, tunable, multi-target and programmable, with fewer off-target effects compared to traditional RNAi or gene knockout methods.

The platform is highly specific. Off-target effects have long challenged classical gene silencing approaches, primarily due to two factors: misincorporation of the passenger (sense) strand into the RNA-induced silencing complex (RISC), and supraphysiological dosing of siRNA or shRNA. Our gene silencing platform addresses both issues.

siRNAs and shRNAs rely on short double stranded RNAs, one of which is complementary to the intended target. This is the antisense, or guide strand. The other has the same strand orientation with the target, or the sense, or passenger strand. Physiological RNAi is highly specific due to preferential incorporation of the guide strand into RISC – the effector complex that triggers target silencing, while the passenger strand is normally degraded. However, synthetic siRNA and shRNAs often show poor discrimination between guide and passenger strand, leading to unintended silencing of targets with full or partial complementarity to the passenger strand. By contrast, our platform uses the cell's own miRNAs which are minimally edited and maintain high preference for guide strand incorporation into RISC.

Another major source of off-targets has been supraphysiological dosing. Overdosing of siRNA oligonucleotides and expression of shRNA transgenes driven by strong promoters, overwhelm the RNAi machinery, disrupting endogenous miRNA function and global gene

expression. siRNA and shRNA lack inbuilt mechanisms to ensure the dose does not exceed the tolerance of the cell's RNAi machinery. Consequently, they can have unacceptable levels of toxicity. Because our platform sRNAs are expressed from endogenous miRNA genes, their expression never exceeds the physiological levels of endogenous miRNAs, so they do not disrupt the RNAi machinery.

Our technology also enables consistent, reproducible silencing. siRNA is intrinsically transient and requires continuous re-dosing. A typical shRNA transgene includes multiple gene regulatory elements such as promoters and enhancers, and depending on its genomic location, it can clash with the local epigenetic landscape, often leading to variegated expression. By contrast, our platform relies on minimal edits limited to the hairpin region of the endogenous miRNA gene and, crucially, does not insert exogenous regulatory elements. Therefore the locus continues to be transcribed exactly as before the edits were introduced, and the new sRNA retains the physiological expression programme, which is highly reproducible and predictable.

New and innovative features

Besides solving old challenges of traditional RNAi, our technology also brings new and innovative features, the most distinctive of which is the ability to programme gene silencing. There are nearly three thousand miRNAs annotated in the human genome, many of which can be leveraged through our platform. Some have broad expression patterns across multiple tissues and cell types, while others are expressed in a very cell type- and cell state-specific manner. These expression programmes are highly robust and reproducible, so individual miRNA genes with the desired pattern of expression can be selected to drive the expression of our platform sRNAs. Thus, silencing activity can be induced 'at the right time' and 'in the right place', for example at a particular stage in cellular differentiation, a distinct cell or disease state; and can be sustained or reversible. Moreover, because silencing is achieved through RNAi, it can be calibrated to tune the expression of the target to a desired level rather than completely silencing it. Ultimately, this approach allows precise regulation of a target to the desired level, within the desired biological or therapeutic context.

This degree of target regulation is not possible using gene knockout approaches, which cause complete and irreversible target inactivation. While transgenic shRNA can support cell type-specific expression, this requires the development of synthetic promoters for each desired application, and their expression dynamics can be difficult to predict. Notably, synthetic promoters often struggle to navigate multi-stage development processes such as differentiation of iPSC-derived cells, and result in variegated expression or complete inactivation. Our platform provides instant access to hundreds of endogenous miRNA loci encoded in the genome, each with its own precise and reproducible programme of expression that is homogeneously executed in every engineered cell, and does not require the generation of synthetic regulatory elements.

Another powerful feature of Laverock's technology is the ability to multiplex gene silencing. It is normal for multiple

miRNAs to be produced from an individual miRNA gene, with numerous examples occurring in the human genome. Inspired by this, we have developed our technology to allow expression of multiple sRNAs from the same endogenous locus, thus silencing multiple targets simultaneously through a single gene editing event. This enables complex multipathway cellular engineering and has significant safety advantages compared to gene knockout approaches, which require multiple guide RNAs (gRNAs) to target different loci, increasing the risk of undesired mutations and chromosome abnormalities, particularly in the case of nuclease-based editing. By contrast, Laverock's approach uses a single gRNA to edit a single locus and hence the risk of off targets is much lower.

Therapeutic applications

Programmable gene silencing has broad applications in cell and gene therapy for a series of indications, including oncology, regenerative medicine and metabolic diseases, so Laverock's platform technology is well placed to underpin a wide range of therapeutic products.

Laverock's product pipeline is currently focused on oncology, deploying its technology to overcome the main hurdles faced by cell therapies for solid tumours. CAR-T therapies have transformed outcomes in certain blood cancers, but these successes have not yet been replicated in the treatment of solid tumours, which account for the majority of cancers.

To adapt CAR-T therapy for solid tumours, multiple 'armouring' strategies are being pursued – including genetic modifications to help T cells overcome the hostile tumour microenvironment (TME). A crucial requirement is the ability to block several immunosuppressive pathways at once. Achieving this via gene knockouts demands stacking multiple edits (one per pathway), which significantly increases safety risks and manufacturing complexity. We believe that Laverock's technology provides a safer and more straightforward approach through multiplex gene silencing, enabling silencing multiple factors simultaneously through a single genomic edit.

Moreover, the ability to programme silencing activity brings additional efficacy and safety advantages. Permanently inactivating T cell checkpoints (via conventional knockouts) can impair CAR-T cell function and pose safety risks. Instead, Laverock's approach takes advantage of miRNA expression differences between resting and activated T cells to create CAR-T cells in which checkpoint pathways are dynamically switched off during active immune responses and then restored when the cells are at rest. We find that this strategy produces CAR-T cells with better proliferation and tumour-killing activity than those with permanent checkpoint deletions, and which are less likely to trigger systemic inflammatory side effects such as cytokine release syndrome.

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