PeptiGos, a Next Generation CPP Platform

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Last September, the first cell-penetrating peptide ("CPP") modulated therapeutic, DAXXIFY™ (Revance Therapeutics, Inc.) was approved by the FDA. Like many "coming of age" stories in the biotech industry, the first therapeutic use of CPPs has taken almost 40 years since they were first described. But Sutura believes that it has a technology which, can now build on this first success to become a staple for the delivery of the next generation of nucleic acid and macromolecular therapeutics.

CPPs are short positively charge peptides (5–30 amino acids long) that have enhanced ability to pass across the cell membrane. Enticingly it seems that CPPs can also chaperone a wide variety of conjugated "cargos" in the process. In DAXXIFY's case the highly positively charged 35-amino-acid is formulated with the 150 kDa "botulinum toxin type A" resulting in enhanced efficiency. However, different CPPs appear to have quite pronounced and varied delivery characteristics, raising the possibility that in future CPPs might be tuned to deliver a range of macromolecules both with greater efficiency and specificity. But it's not all black and white in the nanoworld: Not all CPP-mediated mechanisms result in perfect or even safe and predictable outcomes. It has been this inability to understand and harness these characteristics (and the associated regulatory risks), rather than their undoubted ability to deliver their cargo, which has slowed the use of CPPs to date. I chatted with Dr Wouter Eilers, Principal Scientist R&D at Sutura Therapeutics, about the current state-of-the art and the positioning of the Company's PeptiGo technology in the evolving world order of macromolecular delivery.

So, why is delivery important?

We're now in an era of precision medicine where macromolecular compounds such as proteins, peptides and nucleic acids are being developed as treatments for diseases that have remained beyond the reach of small molecule therapeutics.

But new technologies come with new problems. Cells have a multitude of very effective defense mechanisms to keep foreign genetic material and other harmful macromolecules out. The delivery of a therapeutic nucleic acid-based therapy therefore requires that these defenses are disabled. But these are very bioactive molecules and if the technology is too broad or non-specific it can result in unacceptable safety issues.

Furthermore, these macromolecules have poor *in vivo* stability and limited cellular uptake; so they are often given at very high doses. These are large molecules which accumulate in tissues like the liver and kidneys, so high doses can result in high toxicity. And, when you are working with nucleic acids costing in the order of $10,000,000/kg as Sutura is, then poor bioavailability also comes with additional economic costs.

So, delivery requires developers to constantly weigh the benefits against the risks to come up with a product that's both approvable and affordable. It's an equation that, even today, most often results in failure.

What can be done about it?

There are several solutions which have been tested over the years and their use depends on the type of therapeutic macromolecule. There is currently no "one-size-fits all" solution. The simplest approach is to administer a protein or nucleic acid which has been modified in way that improves its stability and hope that enough of it ends up in the target cells. The alternatives are to 'package' the therapeutic in a vesicle such as a lipid nanoparticle (LNP) to protect it and aid its uptake by cells, or to use a modified virus to deliver a therapeutic gene, in case of a genetic disease.

So, what are the drawbacks?

Well, it's difficult to speak in generality as there are lots of different technologies and applications, but in the specific applications that Sutura is looking at [parenteral administration], most products are delivered by LNP. The main drawbacks are that LNPs are non-tissue specific; and they tend to accumulate in the liver and spleen where they can cause toxicity. So, they are less suited to delivery to other tissues and that's where a lot of the industry's interest lies right now.

So how might CPP's help?

Well, we believe that CPPs might provide a means of improving the entry of macromolecules to the cell interior. CPPs are capable of delivering proteins, peptides and nucleic acids, and are relatively easy to produce at a large scale.

However, it's a nuanced field. Some earlier generations of CPP technology used highly charged peptides, which are very effective at entering cells, but do so by disrupting the cell wall; knocking down the front door, so to speak. So, they are often non-specific and can result in tissue toxicity. We believe that the next generation CPP's, such as the PeptiGos developed at Sutura, may benefit from a more nuanced approach, by using a "key" to unlock the "door" into a cell.

So, what is a PeptiGo and how does it differ from a CPP?

A PeptiGo is simply a peptide sequence which has been chemically linked to a macromolecular cargo. This sequence represents the "key". While it sounds simple, there's a lot of chemistry to manage here and only a very narrow difference between "unlocking the door" and "knocking it down". But we've now created a library of PeptiGos which appear to have the right balance and mark an improvement over earlier generations of technology.

So, can you expand on that – how do PeptiGos differ from the other CPPs?

Unlike other CPPs, we believe that our PeptiGos carry the key to the active (energy-dependent) mechanism of cell entry called "endocytosis" – this is the "door" that we use to get macromolecules into the cell safely and effectively. Because endocytosis requires exertion on the cell's part it is thought that this mechanism also confers a degree of selectivity on the molecules that they take in, only allowing those molecules into the interior that they "choose".

Therefore, in order to open the door, the PeptiGo needs to mimic a natural "key" molecule which is recognised by the cell. And this is where PeptiGos diverge from previous generations of CPP which comprise peptide linear sequences: In biology bioactive peptides are actually folded into complex three-dimensional secondary structures. It's this 3D shape that the cell recognises. Simply having the right sequence of peptides is unlikely to suffice as a key to unlock the door.

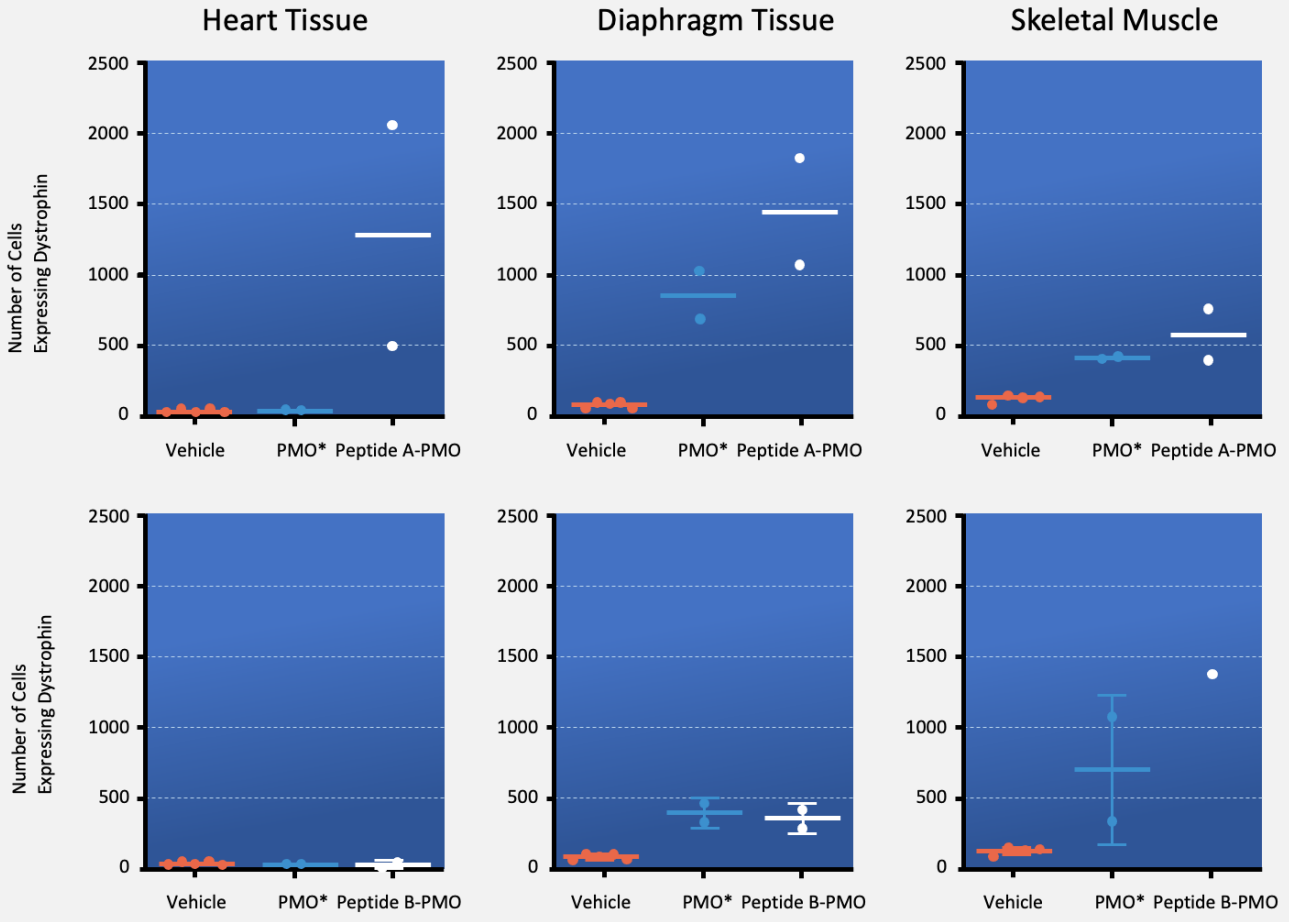
Our patented platform generates bioactive PeptiGos that mimic these folded 3D structures. We use chemistry to staple and stitch the molecules into rigid molecules that closely resemble those that the cell encounters in nature. These molecules also have the added advantage of being more stable and resistant to breakdown, giving them longer in the body to reach their target.

Because different cells recognise different keys. It is thought that our approach may also present opportunities to use different keys to target cargos to specific cell types. And herein lies Sutura's principal advantage.

You mention tissue specificity as being an advantage for PeptiGos can you elaborate?

So, every PeptiGo is different, so it's hard to generalise. But [diagram 1] shows one of the original *in vivo* (mouse) experiments that Sutura was founded on. The experiment used two different PeptiGos: two different Peptides conjugated to the same chemically modified antisense molecule called a phosphorodiamidate morpholino oligonucleotide - or "PMO", which provides exon skipping in a model system using the protein dystrophin. We compared the PeptiGo to the PMO alone, remembering that the PMOs are already used in FDA-approved products. These data show that in almost every instance, the PeptiGo was demonstrably better at delivering the PMO. But perhaps more enticingly, the two different peptides had very different specificity for the tissues that we were looking at.

Diagram 1: Comparison of two PeptiGo constructs vs. the "naked" PMO in selected tissue samples



* Phosphorodiamidate morpholino oligomers (PMO)

Source: Sutura Information

So, what next for Sutura?

Well, there's a growing sense in the company that CPPs provide an eloquent solution to an age-old problem. We are in the process of developing our understanding the role that PeptiGo structure plays in accessing specific mechanisms of action. In parallel, we are also running experiments that will expand our understanding of the associations between PeptiGo structure and their activity in different cell types, which will help us to better organise our chemistry toolbox. We are already in discussions with several companies seeking to enhance the performance of their assets and we are looking for additional use cases around improved cell entry and intracellular trafficking to the nucleus; better biodistribution and improved pharmacokinetics and tissue-specific delivery.