

(1) Can you elude to the conjugation chemistry? or at least describe whether this is site selective? why did you choose full antibody versus fragments?

Dr. Doppalapudi: We used random cysteine conjugation method and it is site selective to mAb hinge region cysteines. We chose full length mAb over fragments because of PK and favorable biodistribution. Conjugates of antibody fragments show faster clearance than full length mAb conjugates.

(2) So does it require repeated injections for DM1 therapy?

Dr. Doppalapudi: Yes.

(3) For the DM1 therapy, dose reduced mRNA levels correlate to improved muscle function in the cyno study?

Dr. Doppalapudi: The cynos in that study were healthy. Unfortunately, there are no DM1 disease model cynos available.

(4) Do we necessarily need to use dynamic light scattering devices for viewing Zinc nanoparticles?

Dr. DeLong: we certainly do use this classic technique to characterize RNA interactions to nanoparticles and have the Malvern Zetanosizer in the lab which besides size, also measures the zeta potential which will typically undergo an anionic shift when complexed to RNA.

(5) You have worked with mRNA therapeutics. What are the strategies you use to stabilize the RNA, and what strategies do you use to minimize the immunogenicity of RNA, or improving the efficiency of translation?

Dr. Cooke: This is a great question. The stability and the translational efficiency of the mRNA is enhanced by using a 5' cap as well as modified nucleosides, such as pseudouridine and 5methylcytosine. These modifications also reduce the cell autonomous activation of innate immune pathways through pattern recognition receptors such as toll-like receptors. In addition, stability and translational efficiency can be enhanced by using a long poly A tail (most of our constructs include a polyA tail of about 150bp). Furthermore, in designing the construct, you want to avoid a lot of secondary structure in the 5' end that can slow translation, as well as rare codons.

Another approach is to circularize the mRNA, in which case it is less susceptible to exonucleases. Whereas linear mRNA lasts minutes to hours, circular RNA lasts for days in the cell.

One can also increase stability of the mRNA in storage by lyophilizing it. Because water rapidly degrades mRNA, removing water markedly enhances stability. We have shipped our lyophilized mRNA internationally at ambient temperature with no significant degradation.

(6) How long is the duration of action of siRNA usually? >> than 12 weeks? How is siRNA then eventually cleared from the cell?

Dr. Doppalapudi: The duration of action of siRNAs will depend upon the in vivo stability of siRNA. Yes, some siRNAs reported in the literature have shown duration of action longer than 3 months. The siRNAs are eventually digested into smaller fragments by endo and exonucleases in the cell and cleared.

(7) Can you measure the therapeutic siRNA in cells released from the lysozyme? How does the siRNA PK correlate to the observed functional KD?

Dr. Doppalapudi: Yes, siRNA loaded into RISC could be measured using RISC loading assay. siRNA PK and biodistribution to target tissue are key factors in the observed mRNA KD.

(8) Can the drug delivery approach using peptide conjugates compete with the NP and the antibody approach or are there drawbacks?

Dr. DeLong: Our lab has explored conjugates of cell penetrating peptide, RNA binding and anticancer peptides. Drawback is if these are recognized as “foreign” and mount immune response against.

Dr. Zhou (CAS): It certainly worthwhile trying. There are cell surface receptors which use certain peptide as a ligand and then internalize it. The drawbacks are that peptides are more subjected to in vivo degradation than antibody or NP if there is no modification on it. Sometimes, people will fuse the peptide with another small antibody fragment to escape the attack from immune system and prolong the half-life in body.

(9) Endosomolytic small molecules with application in ON delivery are reaching optimization. How soon will these types of cotherapeutics reach the clinic with oligonucleotides?

Dr. Zhou (CAS): Conjugating oligonucleotides with endosomolytic small molecules is a very smart way for drug delivery. It is very specific and highly efficient. Tumor cells are usually more susceptible to small molecule induced membrane dissemble. In terms of how soon reaching to clinic study/trial, it is hard to predict since there are many non-scientific factors which likely to be involved as well.

Dr. DeLong: My post-doc advisor Rudy Juliano has started a company on these (Initos Pharmaceuticals | Optimizing the Therapeutic Potential of Oligonucleotides). My guess is they will partner with big pharma to bring these to clinic.