Steps to the Clinic with an mRNA Compound at TriLink

TriLink is uniquely situated as your partner for mRNA drug development and synthesis. As a commercial leader in the research and synthesis of novel modified nucleic acids, TriLink has led the way since the 1990's in mRNA research and development. We were the first to introduce commercially pseudouridine triphosphate, 5-methylcytidine triphosphate, and many other compounds that have made the prospect of mRNA as a systemic drug even more possible.

We have continued to push the boundaries of nucleoside chemistry with the addition of dozens of novel NTPs over the last few years. These new products enable scientific exploration of the interaction of RNA with life. The development of drugs based this research continues to advance the science to new frontiers. We have also heeded our clients' calls for help and continued to develop our ability to manufacture mRNA at increasing scales and increasing purity as their research continues towards the clinic. All this work culminates to the synthesis of clinical grade mRNA, which TriLink also offers.

Taken together, TriLink has the services and capabilities to be your partner from the technical inception of your mRNA research program through the clinical synthesis of your drug candidate.

TriLink is your mRNA Partner

We know that your drug program is key to your company or division's success. We also know that every program is largely unique in need for support and direction in reaching the clinic. In order to better address your program, TriLink has developed a standalone GMP division committed to helping you reach your goals. The management and direction of the sales, quality and manufacturing wings have merged to give you an organized approach to streamlining the process development efforts needed to successfully achieve your final requirements.

As soon as you let us know you are interested in the synthesis of clinical grade material, you become the client of the GMP group. For the Research Phase, you will remain working with the customary service team for research grade materials. However, the earlier the GMP team is involved the better, since they can help you steer your research into clinically viable directions. For the many possible mRNA manufacturing routes important considerations are synthesis scales, purification options and price points.

Once you are a GMP client, your program will be monitored and you will be offered guidance on how to best reach the next Phase in a timely fashion based on your objectives. We will have regularly scheduled meetings on both the technical and business aspects of your program as frequently as needed to ensure our mutual success.

Phases of an mRNA Drug Development Program

There are four distinct Phases to development of an mRNA drug, or any drug for that matter, leading to a Phase I clinical trial. (Figure 1)

1 Research
2 Process Development
3. Pre-Clinical Grade
4. Clinical Grade

Research Phase

TriLink is the commercial leader in the development of novel chemistries for mRNA synthesis. We also have an extremely strong biological research team devoted to the exploration of both modified mRNA and the process of synthesizing better quality mRNA. Both of those resources are available to you in the development of your mRNA drug candidate. We have found several critical areas that require your attention and for which we offer alternatives depending on your individual need.

1. Sequence Selection

The sequence you choose as your target mRNA has a number of very important ramifications. Any unusual structure will make the compound harder to make and analyze and should be avoided if possible. The base composition of the primary sequence is also critical. We have found that uridine depletion of your sequence has a number of beneficial qualities including access to a larger range of modified uridines and a general reduction of immuno-stimulatory properties.

2. Plasmid/Untranslated Region (UTR) Selection

The vector used to produce the template is very important. The restriction enzyme cutting sites need to be carefully vetted. Ideally the enzyme should cleanly cut the plasmid at a single location, leaving an overhang in the correct orientation, and be inexpensive to keep large scale production costs down. The 5' and 3' UTRs needs to be designed with uridine content depleted as well, and with no undesired cutting sites. TriLink offers an entry vector with the UTRs already embedded as well as an 80 length polyA track templated.

3. PolyA Tail

To incorporate a longer tail, TriLink can incorporate a fixed length 120mer tail to the template through PCR, or we can use polyA polymerase to add a longer tail with the disadvantage of yielding a range of lengths, not a discrete length. Both methods have pros and cons. The polymerase route is expensive, where the PCR addition of the polyA tail is limited in its ability to scale up. Our technically savvy research grade sales team will be able to discuss the differences with you.

4. N7-Methyl-Guanosine Cap

We have the ability to add the 5' cap through a number of routes. The most viable for a clinical program is either enzymatic capping done post-transcriptionally or TriLink's own CleanCap™ technology, which is a co-transcriptional capping reagent. Both yield cap 1 structures and both yield 90%+ capped material, possibly nearing quantitative capping if either approach is optimized. The main difference is that CleanCap™ is about half the cost of the enzymatic approach, which could lead to millions of dollars in savings *per batch* by the time you reach the clinic. We highly recommend you make the move to CleanCap™ early in your research program.

5. Base Selection

The decision to use either wild type (WT) or modified bases is in many ways tied to your drug candidate. Is the treatment *in vivo* or *ex vivo*? What cells are targeted? What is the method of delivery? Are you seeking immune stimulation or wish it to be reduced as much as possible? All these play a role in base selection. In some cases all WT bases coupled with more extensive purification is all that is necessary. In other cases you may be able to

find modified bases that reduce the immune stimulatory properties of your specific compound to the level where very little purification is necessary.

TriLink offers more than 40 modified NTP's that can be used for the exploration of immuno-stimulatory properties. We have found that the proper base is highly dependent on your sequence and application. Furthermore, there are intellectual property (IP) issues surrounding several of the more popular modified bases. We offer many modified bases that are not impacted by IP, so hopefully one will suffice for your needs. In order to determine which base is best there is only one route - try as many as you feasibly can. We offer a series of suggestions in a separate document entitled "How to Select the Best Modified Base for My mRNA". We recommend you try to find an IP free modified base or see if WT bases will work. The benefits to your overall program as you approach the clinic are obvious. Our technical team will be able to help you decide what modifications to try, although the problem seems to have different solutions for different clients.

6. Method of Purification

At scales less than 40 mg, we use silica based columns for the initial clean up to remove proteins and other contaminants. At larger scales we move to our LC Isolation™ method which is very effective at removing the transcription reagents and many of the impurities generated in the reaction. For some applications LC Isolation™ is sufficient, even with WT bases. Modified bases can greatly reduce the immune stimulatory properties of the mRNA and any side products still remaining, such as double stranded RNA (dsRNA). They may negate the need for further purification in some cases.

If further purification is needed, we offer a RP-HPLC purification process that is effective up to approximately 250 mg crude mRNA. We are actively working on further scaling of the method. At the current scales we can often reduce the dsRNA considerably, and depending on the purity required, are able to remove truncated mRNA sequences as well. We have learned that the optimization of the RP-HPLC method is also a compound specific problem, and must be developed for each individual compound after the final design is chosen.

7. Capping Assay Development

Percent of capped product has a direct correlation to the functionality of the mRNA. At TriLink we believe that having a capping efficiency assay will be important from a regulatory perspective for release of clinical grade products. If you opt to use our entry vector, the good news is we have a capping assay developed. A product specific qualification program will still be required. For your own 5' UTR, we highly recommend having us develop a capping assay specific to your compound if you do not have one already. This should be started early in the program.

Process Development Phase

Once the design of the compound has been defined through the Research Phase, the next step is to scale up the synthesis. If your trial is small and we have already made the required amount of the material for you during the Research Phase, Process Development Phase can be omitted. We will move directly to the next Phase, Pre-Clinical Grade. However, there are a few business aspects that need to be address in the Process Development Phase, whether you need to scale up or not. For example when you take into account the amount needed for analysis and stability testing, you may find you need much more material for your trial than you thought.

Please read this section even if you require a small amount of material.

Your program will have the personal attention of the GMP division who will help you track your program each step of the way to reach the successful conclusion of your clinical synthesis.

1. Scale-Up

This step happens once you have determined the exact construction of your drug candidate and we are ready to attempt it at a larger scale. Often times at this point the purification method will need to switch from silica membrane to LC Isolation™. We expect to see no change in mRNA performance when switching between these two purification methods. The importance of the scale up process is to demonstrate that the transcription and purification at this larger scale, generates the same quality of mRNA as the Research Phase of the program.

2. Quality Assurance

During this Phase your Quality Assurance (QA) and TriLink's QA teams need to meet to start the process of designing a cGMP Project Order. The Quality Agreement should be started and an audit scheduled. Raw material specifications need to be set. Types of analyses needed chosen.

3. Reserving a GMP Lab Slot

If you know your time schedule, now is when the slot should be reserved for your first synthesis. The reservation fee will be fully credited towards your synthesis of your clinical grade material. TriLink will remain as flexible as possible if the reserved time slot needs to shift due to matters outside your control, but it does ensure that you have a place in our rapidly filling queue.

4. Negotiation of the Service Contract

The price of this and the next steps are considerable by any measure, and is highly dependent on the choices made in the Research Phase when designing the drug composition and manufacturing process.

We are flexible in pricing based on the amount of risk you are willing to take. The raw materials can be very costly, but if you cover them *at your risk*, you pay only the cost of those reagents with shipping, plus the costs of QC/QA testing and handling them at TriLink. *At your risk* means you will replace the materials if the synthesis has to be repeated. If it is at TriLink's risk, we have to charge a margin sufficient for us to accept that risk. Other costs that are incurred through cGMP synthesis include labor and facility overhead. All labor fees are based on standardized per hour costs for the personnel that will be performing the identified tasks and/or processes. TriLink utilizes a set per day GMP facility fee to cover all GMP facility overhead.

By streamlining the manufacturing process during the Research and Process Development Phases you can realize significant cost savings in the Pre-Clinical and Clinical Grade Phases.

Pre-Clinical Grade Phase

It is time to ensure that the manufacturing process chosen for your compound is compatible with the amount needed for first clinical batch and complete the appropriate documentation for the Clinical Phase.

1. Scale-Up Continuation

If the yield in Process Development Phase has not met or exceeded the amount needed for the Clinical Grade, the scale up process must continue. The manufacturing scale for the Clinical material must be done at least once outside of the cGMP facility for a Phase I trial.

2. Documentation

Raw material release specification documents should be finalized. The Quality Agreement should be executed. The Master Batch Records will be started as drafts and be living documents during this Phase.

3. Release Specifications

The release specifications should be tentatively set with final specifications determined after the Pre-Clinical batch is done. Assay qualification should be completed in parallel with defining release specifications.

4. Confirmation of Slot

The slot in the queue should be finalized and payment for slot reservation should be received.

5. Conclusion of Quality and Service Agreements

Both of these Agreements should be executed before proceeding to Clinical Grade Phase.

Clinical Grade Phase

By now all the issues should have been dealt with and we are merely pushing the start button.

1. Documentation

Release specifications should be set and Master Batch Records need to be approved by all parties prior to start.

2. Delivery

If all things go as planned, you should receive your clinical grade mRNA on schedule and on budget. All the hard work by everyone will pay off at this time and we will be waiting for your next drug in the pipeline.

Summary

Hopefully this introductory document gave you an understanding of how a partnership with TriLink will help you navigate the launch of a novel mRNA therapeutic. Although we do not have all the answers, we have many, and are continuously building our knowledge base. Together, we have much to learn and many lives to save.

For more information please contact sales@trilinkbiotech.com