EXPERT ROUNDTABLE

Driving the expansion of mRNA into the therapeutic sphere

Elisa Manzotti, CEO of BioInsights, speaks to **Alejandro Becerra,** Thermo Fisher Scienitific, **Andreas Kuhn,** BioNTech, and **Metin Kurtoglu,** Cartesian Therapeutics

The advanced therapies industry is heavily engaged in capitalizing upon the extensive 'proof of concept' gained through the success of mRNA-based COVID-19 vaccines. Novel therapeutic applications in major disease areas, including oncology, continue to show promise in preclinical and early clinical studies, yet challenges remain.
Here, a panel of thought-leaders from the mRNA field will consider the ever-expanding reach of mRNA technology, exploring at a high level how and where it will impact the advanced therapies space moving forward. The panel will then dive deeper into specific trends, issues, and innovations in mRNA processing (particularly downstream) and analytical development, discussing key areas for improvement and corresponding solutions.

ALEJANDRO BECERRA is a Principal Applications Scientist and Global Purification Technical Lead. Alejandro has over 15 years of experience in downstream processing and customer support having worked as Purification Team Manager and other bioprocess engineering roles prior to joining Thermo Fisher Scientific in 2018. Dr Becerra is a subject matter expert in preparative chromatography with expertise in the development, optimization and scale-up of antibody, recombinant protein, and viral vector purification processes. Alejandro holds a PhD in Chemical Engineering from Cornell University.

ANDREAS KUHN has worked with RNA for almost 30 years. This started with his diploma and PhD theses on the structure and function of small non-coding RNAs using biochemical and molecular biology methods. In his post-doctoral work, Andreas studied RNA-protein interactions in the spliceosome in yeast and later worked on small molecules to affect pre-mRNA splicing. His work on mRNA-based immunotherapies began in 2007 in the academic group of Ugur Sahin at the University Clinic Mainz, and Andreas joined BioNTech SE shortly after its founding in 2008. In his current role as Senior Vice President RNA Biochemistry & Manufacturing, the main focus is expanding proprietary technologies to increase the efficacy of mRNA-based therapies and to develop and optimize GMP-compatible manufacturing processes and analytical methods for RNA. He has co-authored numerous publications and patents ranging from basic research on RNA to its application as a therapeutic agent and vaccine.

METIN KURTOGLU is a medical oncologist board certified in internal medicine. Dr Kurtoglu's clinical and basic science research career spans over 20 years and has focused on developing novel targets for drug-resistant cancer cells and cancer stem cells, including multiple myeloma. He has also been an investigator in various cancer immunotherapy trials. Cartesian Therapeutics is pioneering RNA cell therapies in and beyond oncology, with three assets in clinical trials for autoimmune, oncologic, and respiratory disorders. The investigational therapies are manufactured at Cartesian's cGMP manufacturing facility.

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Q What have been the key R&D directions for mRNA and associated technologies, as they expand beyond prophylactic vaccines?

AK: Let me start with stating that while it is often thought that using mRNA was invented for developing the prophylactic vaccine against COVID-19, there had previously been several years of basic and applied research performed with mRNA. This work was the basis for speeding up development of the COVID-19 vaccines.

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– **Metin Kurtoglu**

A related approach to mRNA-based infectious disease vaccines is using mRNA to activate the immune system to kill cancer cells in a therapeutic setting. With the same goal, mRNAs are also used to encode proteins such as antibodies or cytokines, which for me, is a key application area. On the research side, there are two main areas of development: firstly, the mRNA molecule itself, and secondly, the formulation, which is where lipid nanoparticles come in. More development in formulation is needed, as it will be key for the field to move beyond the liver into other tissues – intramuscular delivery for the prophylactic vaccines was very important.

While there are already efficacious vaccines on the market, there is an opportunity to further improve the mRNA molecule itself – for example, lowering dose and improving tolerability are two key areas if we are to successfully move beyond prophylactic vaccines.

MK: Another exciting current event in the field is the expansion of mRNA therapeutics into new indications. Because mRNA is now used for vaccination purposes on a global level, people start to get more comfortable with using mRNA elsewhere – for example, in autoimmune disorders or other internal medicine diseases. Secondly, in terms of mRNA delivery, one very interesting solution in development is delivering mRNA therapeutics in the context of a live cell. In both autologous and allogeneic cell therapy areas, there are some very interesting new technologies going after unique diseases using live cells transfected with mRNA.

AB: Being a supplier of products for the manufacturing of RNA, we are in a position where we can look across a wide range of both smaller and larger organizations and see what they are working on. We observe the movement not only towards vaccines but also to personalized medicine, such as cancer vaccines in the oncology space. With regards to the mRNA itself, there is a big focus on utilizing different approaches to reduce dose, as Andreas mentioned – whether it is with traditional mRNA, self-amplifying RNA, or new molecules like circular RNA (circRNA).

Q What are some of the major challenges that face the field as it makes this migration into therapeutic drug applications?

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– **Andreas Kuhn**

MK: Using mRNA to vaccinate against infectious diseases works really well. The mRNA itself is very immunogenic and the body will immediately react to it. However, when you go after a disease that needs a long-term therapeutic effect, it will be challenging to produce the right type of RNA in a formulation that results in sustained therapeutic activity. The greatest challenge in moving beyond infectious diseases and into the therapeutic sphere is to come up with less immunogenic solutions. At Cartesian, we are focused on using live cells as a vehicle because the cells protect the mRNA within its physiological environment, reducing immunogenicity.

AB: From the development perspective the purity of the mRNA is critical, and closely associated with purity are the analytical challenges. A purification process is only going to be as robust as the analytics that are available to develop it. It will be critical to establish better methods in order to characterize the product-related impurities. Next to this technical aspect, another challenge in continuing to develop technology is having the knowledge and expertise available in all parts of the world – building a skilled workforce with the requisite training provision is important.

AK: Less immunogenic and purer mRNA is not going to be sufficient for delivering success in advanced therapy applications. With vaccines, only relatively small amounts of protein are needed in order to obtain a huge amplification by the immune system. On the other hand, using mRNA for the expression of functional protein requires several orders of magnitude higher expression of that protein. Therefore, looking into improved expression of the mRNA is key – for example, through improved sequence design.

In addition, I would like to reiterate the importance of delivery. With the current methodologies, it is often that only a small amount of the injected mRNA ends up in the target cells. Being more efficient in delivery and formulation is another major challenge the field needs to overcome if it is to open up all the opportunities which mRNA as a therapeutic modality has to offer.

Q What will be the key technological/platform developments and innovations required to address these challenges?

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– **Alejandro Becerra**

AB: There are ongoing efforts to improve the purification toolkit for the mRNA field. More specifically, when we are looking at eliminating double-stranded RNA (dsRNA) from the final product, current efforts are focusing both on the *in vitro* transcription (IVT) reaction as well as the downstream process. There are some potential approaches that might be difficult to scale up today, but with the ongoing efforts and collaboration between suppliers and producers of these therapeutics, it will lead to the development of new products and accelerate production processes.

AK: We still have a lot to learn about mRNA. The key is to understand what makes a specific mRNA optimal in the context of using it as an exogenous mRNA. To build more knowledge on bringing mRNA into the cell, we need to understand what happens with the mRNA: how is it taken up by the cells? What factors in the cells are important to translate the mRNA?

Another development is taking some of the technologies that are used for other biological molecules and applying them to mRNA. There is a lot of existing knowledge on purifying biological molecules, including on the analytics side, that can be applied to mRNA. We will need improved analytical techniques to better understand what the molecule is that we have in hand.

MK: Hopefully, the number of products in the RNA cell therapy world will start to expand once we start showing promising data in more indications using therapeutic RNA in the context of a cell. The cell uses the mRNA for the therapeutic function at the right level, because these cells intrinsically know how to express at a level that causes bioactivity. These products are just starting to enter clinical trials and as they are beginning to show proof of concept, we will see more live cell therapy applications using RNA. In this way, the therapeutic RNA activity can be taken to organs by using their physiological pathways, rather than trying to figure out all the details exogenously.

Q Looking at mRNA therapeutic manufacturing, what are the main limitations with the current processing tools and technologies? Where specifically do we need to improve in both upstream and downstream processes, and what approaches will yield this progress?

AK: As we have discussed previously, purification is one area where further improvements can be made. mRNA are highly charged molecules, which causes issues with some of the more standard types of chromatography such as ion exchange chromatography (IEX). New tools are needed to help us to purify mRNA at larger scales – not the micrograms to milligrams of mRNA required for preclinical proof-of-concept studies, but tens to hundreds of grams.

Looking at the IVT reaction itself, T7 RNA polymerase is a well-behaved enzyme in general, but it has some limitations such as creating double-stranded RNA as a byproduct. Developing a broader toolbox with alternative RNA polymerases that have better characteristics will be useful. If the product coming out of the IVT reaction is purer it will put less stress on the purification process.

Another current limitation with the materials is the need to renew the DNA template whenever you produce a new RNA sequence. New technologies to assemble and amplify DNA – for example, in a cell-free process rather than using *E. coli* – would be helpful in quickly getting a DNA template for mRNA manufacturing.

AB: When we look at how manufacturing tools have evolved for other biologics such as monoclonal antibodies, some of the more important advances came through collaborations between manufacturers and suppliers. We should leverage a similar approach in the mRNA field and work on close collaborations to develop the new tools. These joint efforts will get us to the right tools faster.

MK: The design of the mRNA is the biggest challenge in mRNA manufacturing. How much mRNA is needed to make enough protein in order to achieve the therapeutic function? The answer is that the amount of mRNA required depends greatly on the design of the mRNA. If you can design a mRNA where you only need a microgram to give the desired therapeutic effect, then manufacturing is no longer going to be a challenge. The second challenge relates to the delivery system: whether you are a LNP or a cell, the limitation and bottleneck right now is in scaling up of the delivery systems.

Q Can you provide insight into the current practice of process monitoring and optimization of IVT and LNP formulation?

AK: At this point there is to my knowledge no online process monitoring available for mRNA manufacturing. There are some tools for reporter constructs that have a fluorophore sequence element in the RNA, but this is very specific and can only be used to perform generic process development. Right now, we look at the reaction over time by taking samples and monitoring the effect, which is a laborious practice. At some point, when the technology is mature, tools will become available to monitor the productivity and yield of the RNA production process online. Hopefully, we will move into the sort of online monitoring

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that we see today in fermentation processes, where one can respond rapidly and add nutrients when the cells are growing too slowly, for example.

Q Can you go deeper on how the mRNA purification toolkit is evolving to address current challenges?

AB: The main unit operations in purification are the filtration and chromatography steps. We see a significant number of manufacturers utilizing POROS™ Oligo(dT) affinity resins for one of the chromatography steps in the process, whether it is after the IVT and/or after the capping (when the capping takes place post-transcriptionally). In terms of filtration, there can be multiple tangential flow filtration (TFF) steps and of course, a membrane filtration step at the end.

The currently available filtration toolkit can be improved. For example, particularly for smaller companies that are just starting to develop their process, there might be instances where there is a lack of a representative scale-down model. For chromatography and more specifically, with POROS Oligo(dT) affinity resin, I think we are in a good place today, but with the need to purify larger molecules, the binding capacities can be relatively lower. At the moment, Thermo Fisher Scientific is looking into collaborations to investigate different strategies to maximize the binding capacity with commercially available products. Long-term, we are looking into developing more specific base beads or other chromatography supports that can further improve the performance.

The polishing steps have challenges as well – for example, when reversed-phase chromatography is used and there is a need to utilize solvents (particularly an issue at larger scales). There is room for improvement, whether it is through new chemistries, or different approaches and methods that are more good manufacturing practice friendly.

MK: To make an incredibly pure mRNA is always a challenge. One way to circumvent this challenge is to allow some impurities in your therapeutic product that will not impact the outcome whether it relates to safety or efficacy. Choosing a cell as the delivery vehicle will help here, because the cell has built-in mechanisms to eliminate impurities such as nucleoside triphosphates (NTPs) or double-stranded RNA. More interestingly, you could manufacture a cell in such a way as to ensure those impurities are eliminated by the cell during manufacturing.

AK: One thing that can help overcome the lower capacity challenge of chromatography resins is to establish a form of continuous chromatography. Rather than having to increase the column size in relation to your batch size, you could overcome the lower binding capacity challenge by prolonging the process.

Q What are the key areas for improvement in the analytical toolkit?

MK: mRNA is a fairly heterogeneous molecule by nature. For example, the length of polyA is usually not uniform. Finding the right analytical tests to determine the features of these heterogeneous mRNA molecules is challenging.

AK: One of the challenges at this moment is the diversity of methods used to analyze the same parameter. One example is measuring RNA integrity, which indicates the amount of full-length RNA versus the amount of degradation products or truncated transcripts. Analysis of RNA integrity can be performed by using a large variety of techniques and you can question how the results of these different techniques correspond to each other. Harmonization and standardization of analytics is very important for moving forward. A comparable situation is the use of internal standards to measure double-stranded mRNA. Individual companies are using different standards at the moment, which raises the question of how comparable the numbers are.

On the other hand, there is the challenge of the technical limitations that some of the analytics have. Developments are taking place – for example, in the sequencing technology area – that will help us to better analyze samples. More advanced sequencing technologies are emerging, which can improve knowledge of the mRNA molecule itself.

Q Do you expect regulatory guidelines to be set for mRNA and siRNA manufacturing in the near future? If so, how do you see these guidelines impacting the freedom to operate that the field enjoys today?

AK: The European Directorate for the Quality of Medicines (EDQM) has started an initiative to draft guidelines for mRNA therapeutics. Initially, it will be for prophylactic vaccines because these products are already on the market, but this initiative will definitely help in creating guidelines for all mRNA therapeutics. There is already a guideline from the World Health Organization (WHO) and the US Pharmacopeia (USP) has drafted a guidance document as well. Most likely, there will be more to come.

The question around freedom to operate is interesting… When there are no guidelines, people complain that they don't know what to do, but when there are guidelines, they complain that they have to follow them! At the end of the day, regulators usually have good reasons why they ask for certain things, and more regulatory guidelines will clearly help further development of mRNA therapeutics.

MK: Having regulations that are outdated and restrictive is even worse than having none at all. In the field of mRNA that is changing now, due to the COVID vaccines. People have started to differentiate regulations for mRNA from DNA. This is key because all the guidance that existed before stemmed from DNA-based therapies. However, unlike DNA, mRNA is a biologically degradable molecule. DNA lives for years – millions of years, in some cases – but that is not the case with mRNA. Thus, the safety profiles for mRNA are

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a lot stronger than those of DNA-based approaches (which is why allowing certain impurities in mRNA therapeutics may be acceptable, if it does not impact the efficacy). Nevertheless, historically speaking, all the regulations, guidelines and analytical tools have been focused on DNA-based engineering, solely because that technology has been around longer in terms of therapeutic applications. But regulatory guidelines for mRNAs are catching up.

Additionally, the way in which you analyze mRNA that is delivered in a living cell will be very different than how you analyze mRNA that is administered through LNPs. Regulatory guidance will evolve over the next few years as more products using different RNA-based approaches come out. The guidance will need to be finetuned depending on the specific product.

Q Concerning the optimization of current mRNA-LNP formulations, does the panel have any ideas on what is the preferred target in this regard?

AK: If you go beyond vaccines you are going to have different cells that you want to target. In a case where mRNA is used in protein or transcript replacement therapy, when there is a protein missing due to a genetic defect, you must get the mRNA into that specific cell type. The question is, what cells can we reach? The more different formulations you have that can deliver the mRNA to a specific cell type, the more diseases you can tackle. Some cell types will be easier to target. If the field goes beyond and into the brain, for instance, then it will be necessary to find something able to cross the blood–brain barrier, which will be a lot more challenging.

Q What do you see as the most challenging step in the downstream process?

AB: One of the bigger challenges we see, particularly for mRNA therapeutics as opposed to vaccines, is the removal of double-stranded RNA. There has been some success with reversed-phase chromatography, but with the inherent challenges of scalability and using high temperature solvents that we discussed previously. Fortunately, this challenge may also be addressed during the IVT process, so it will hopefully be solved in due course.

Q Does Thermo Fisher manufacture any other bead-based products for purification of mRNA apart from the POROS Oligo(dT) Resin?

AB: We mentioned that there are different approaches and it all depends on the required purity of the initial material. When the final use of the mRNA is as a vaccine, then affinity purification and filtration may be sufficient. But where you need to remove double-stranded RNA and other product-related impurities, you will need alternative methods

such as reversed-phase, ion exchange, and/or hydrophobic interaction chromatography. Thermo Fisher does offer these alternatives. With the latter two, we are still learning together with our customers about whether they will be the right tools for this particular purpose.

Q Finally, can you sum up your visions for how and where mRNA will impact across the advanced therapies field in the future?

AK: Due to the success of the development of the mRNA-based vaccines against the coronavirus, expectations are high right now. As we see with many new technologies, people are overestimating the short-term benefits and then underestimating the long-term effects. It will take time to have the next mRNA product on the market and there will probably be some drawbacks and challenges that the field needs to overcome first. In the situation with the coronavirus, we knew which protein to tackle. The formulation was there, the mRNA was there.

Ultimately, though, mRNA will have a huge impact on medicine in general. Similar to where antibodies are today, mRNA therapies will make up a huge part of the market. The next mRNA products on the market will most likely be prophylactic vaccines to fight other infectious diseases such as influenza, RSV, malaria, and HIV to name a few. The second wave will be in oncology products, especially where similar approaches can be taken as with the vaccines, meaning stimulating the immune system to attack cancer cells. There is promising data coming out already here, especially with individualized approaches where cancer cells are sequenced, and you identify new epitopes that are very specific to the cancer cell. After that, I would say the next breakthrough will either be new therapies to battle genetic diseases, or in the field of antibody- or cytokine-encoding mRNAs.

AB: We may see some of the first new products coming out in the vaccine space. Hopefully, other geographies will have access to these new vaccines, and the focus of these vaccines might be on diseases that are more prevalent in other parts of the world, beyond North America and Europe. In the long-term, we will see more growth in *ex vivo* or gene editing applications of mRNA, in addition to more therapeutic application areas such as monoclonal antibodies.

MK: Managing expectations is important. If you want to go after infectious disease vaccination, mRNAs are immunogenic and work great. The gap to the next chapter for mRNA therapeutic applications is large. The good news is that people have been working on RNA therapeutics since the field was invented in 1970s, and have invested heavily since the 1990s. Hopefully, these 30–40 years of development have given us new insights that will help to close the gap faster. Still, the fact that there has been 30 years of work done on mRNA and yet no product other than COVID-19 vaccines came to market, does points to developing RNA based therapeutics is a challenging task. I am hopeful, though, that new applications will come on the horizon in less than 5 years. I think that what can happen quickly is combination

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therapy through mRNA vaccination, where you will be able to vaccinate people against multiple diseases simultaneously.

There are some new and unique indications in oncology and there are new programs in autoimmune disorders, a completely new field that RNA therapeutics never reached before. There might be unexpected therapeutic indications that come up, too, because mRNA is a very versatile tool. There is going to be a greater explosion in mRNA therapeutics than will be in the DNA-based engineering field over the next 5–10 years.

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AUTHORSHIP & CONFLICT OF INTEREST

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