Roundtable Session 2 – Table Number 21-
Unlocking the Genetic Code: NGS Marvels in the Realm of Cell Therapy

Facilitator: *Chong Wang, Takeda*

Scribe: *Maya Gorgas, Genentech*

Abstract:

Next generation sequencing (NGS) is a powerful tool and plays a crucial role in providing valuable insights into genetic information with its massive parallel sequencing capability. In CMC space, more and more NGS based methods have been implemented to 1) evaluate drug product safety and detection of any potential biological contamination, 2) perform quality control and process optimization based on genetic profiles of starting materials, intermediate products, and final drug products, 3) assess genomic alteration after gene editing or chimeric antigen receptor (CAR) integration. In this session, we will focus the discussions of NGS methods on the following aspects: 1) general introduction of NGS methods demonstrating how NGS works, popular NGS methods and their deliverables; 2) applications of NGS methods in cell therapy including plasmids/viral vector identity testing, adventitious virus detection, integration site analysis for CAR, off-target and genomic alteration analysis after gene editing, transcriptomics profiling for product characterization and CQA finding, etc; 3) considerations during method selection for each application, where cases of NGS method selection and the thought processes and considerations during the decision making will be presented as example; 4) regulatory recommendations, with a spotlight on ICH Q5A (R2) draft guideline for adventitious virus detection, where NGS method is encouraged as a replacement of in vivo assays, which underscores the trend of agencies for promoting NGS assays in drug filing process; 5) current challenges and solutions in data analysis and interpretation, assay standardization, validation, and quality control, etc; 6) future perspectives. Overall, the session will hopefully collect feedback from colleagues in academia, industry and regulatory agencies, and to potentially bring broader adoptions of NGS technologies in the field.

Discussion Questions:

1. What are the NGS based assay(s) are you currently using, or plan to use in the near future, for CMC purpose?

2. What are the application(s) of the NGS based assays in your case?

3. What are the future direction(s) and application(s) of NGS based assays do you envision in CMC field?

4. What are your strategies to solve challenges in data analysis and interpretation, assay standardization, validation, and quality control?

5. What are the other challenge(s) you have encountered using NGS based assays, and what are your solutions?

Notes:

Facilitator Comments (C):
We have a variety of participants here who are interested in applications of NGS, and we have both people interested in benchwork as well as bioinformatic/data analysis today.

Question (Q) #1) What NGS based assays are you currently using, or plan to use in the near future for early stage CMC/research purposes?

Answer (A) #1: Cell therapy analytical development
Two Main Focuses:

1. Supporting the process development and helping the team to figure out CQAs affecting manufacturing process success and drug product potency.
2. Pushing NGS in GXP as required by new ICH Q5A(R2) guidance for viral safety testing.

A #2: Process development for stem cell therapy

1. Initial interest to participate in the roundtable discussion was to learn more about feasible ways to implement NGS in GXP.
2. Current NGS applications are for cell identity post differentiation process, to confirm if the cell differentiation was successful and the purity of the final cell population.
3. Transcriptome analysis was also used to determine splicing events, in order to help with cell identity determination after differentiation.
4. For GXP purposes, currently still using Sanger sequencing; but want to move towards NGS.

A #3: Gene therapy / cell therapy

1. Understanding CAR insertion site and off-target events post gene editing.
2. Research on complicated MoAs, determine how transcription signature can correlate with potency and cell product expansion during manufacturing process

A #4: Analytical development in biologics

1. Using NGS for guide RNA purity determination
2. Off-target events confirmation
3. Using RNA-seq together with proteomics data to confirm findings from each other

A #5: Bioinformatics

1. Has seen clients using NGS in both research and GXP purposes.
2. Exploring possibilities of genome stability?
3. What is the ideal sequencing depth for various purposes?

A #6: Analytical development - Gene therapy

1. Identity testing: Using NGS to find indels and mutations
2. NGS is much more complicated than Sanger-sequencing, with longer turnaround time

Participants Comments: We see that after PCR amplification, many mutations can be introduced by the PCR polymerase. Non-PCR based NGS library prep methods definitely worth

looking into, especially in case of sequencing the guide RNA, which is synthetic, therefore no need to worry about the starting materials amount available for the testing

Q #2): Do you use NGS in-house or outsource? If you use both, which works best? What are the main challenges you encounter utilizing NGS methods?

A #1: Outsourced

- NGS for GXP usage is outsourced
- Sometimes we develop in-house assays and transfer to CRO for GXP validation, this might be the approach we will be using for customized NGS based methods

A #2: Combination

- NGS methods for research purposes supporting process development is in-house
- NGS for GXP usage is outsourced, but is exploring building in-house

A #3: Outsource entirely

A #4: Building NGS in GXP in-house, but there are a lot of hurdles as to how to set up the lab logistics to keep the contamination levels at bay in the lab setting, how ventilation works, how to store and analyze the data (i.e. data infrastructure building), etc

A #5: Data analysis and infrastructure building is a challenge, but still doable

Q #3) What are the methods you used to confirm your findings from NGS assays?

A #1: Cell based methods for confirmation (FACS, etc). We have also used NanoString to confirm the findings from NGS, and turned out to be very comparable.

A #2: For off-target events, we use computational prediction for finding off-target sites, and use NGS for confirmation.

A #3: FACS and RT-PCR based methods for confirmation.

C: For GXP: The FDA is pushing NGS into the GXP space with the finalization of ICH Q5A, which is the first use case of the NGS-based method in GXP space.

***Participant comment/question*: In some cases, NGS is used for identity testing to capture mutations/indels in a given sequence. Is NGS based method a qualitative method, or quantitative method in your cases?**

A #1: Qualitative. It is important to set up a criteria to eliminate background from the sequencing errors. But in general, NGS is used as a qualitative method.

A #2: We are exploring the possibility of using it as a quantitative method. The reason is that NGS is with higher sensitivity and accuracy, it might be better to determine the impurity. We are also thinking that since the agency is leaning toward NGS based methods, we can multi-purpose our NGS assays.

A #3: Why not just use q-ddPCR for quantitative analysis, and NGS as a qualitative method?

***Participant comment/question*: For adventitious virus detection, what is the proper way to set up the control? Is it crucial to detect non-replicating viruses?**

A #1: Our approach in planning is to detect both active replicating viruses and non-replicating viruses by preparing NGS libraries from both DNA and RNA extracts. We don't know how sensitive the assay will be, or how much background we will end up seeing.

A #2: For detected DNA, it might not be an active virus, it could be only a contaminant. More development work is needed to establish the data interpretation standards - if a certain sequence from a virus is detected, how do we interpret the results?

A #3: Agree with the points that we need to set up a criteria or standard as to how to interpret the data.

Facilitator conclusion:
Thank you everyone for participating in today's discussion. Our productive discussion covered the majority of the aspects originally planned to discuss, with more questions raised during the conversations. NGS is a relatively new technology and new topic to the agency perspective, it is understandable that there are still open questions in the field. We will keep exploring and hopefully getting more guidance from the agency, and thanks so much again for the discussion!

.