**Journal Club:** GeoMx DSP

**Slide 1:**

Hello everyone, before we start the journal club, first, some people here might wonder why I choose to talk about GeoMx. To put this into context, in the MODICED project, we expected to receive spatial proteomics data generated from this platform, that I would subsequently analyse, but we are still waiting for the data to come out for technical issues. So we don’t have results to present yet, but I thought it would be interesting to give you an overview of what we can do with this technology, of how it works, and of the type of results we can pretend to get at the end.

**Slide 2:**

Okay, so let’s start, so essentially with this platform, we can do both gene and protein expression profiling from bulk tissue samples, while keeping the spatial information.

But you might wonder here, how is it possible to retrieve quantitative profiling data from a single tissue biopsy ?

In fact there are 5 steps required to achieve this, first, onto a single tissue section, whether it is FFPE or fresh frozen, we combine two types of reagents, the imaging reagents used to image or stain your tissue, and the profiling reagents to profile it .

The imaging reagents can be either fluorescent antibodies for protein and RNAscope probes for mRNA. And the profiling reagents, are either antibodies or ISH probes which are tagged with oligos via a UV-photocleavable linker.

Once the sample preparation is done, the slide will go onto the GeoMx system, to be scanned.

After that, using the fluorescence of the resulting image, the user can select the regions of the tissue he wants to profile.

And for each region selected, a digital micromirror device integrated to the platform will shine a UV light. This will release the oligonucleotides that will be aspired by a microcapillary tube , which in turn deposit them into a 96-well plate, where each well is indexed.

After we went through all the regions selected, the oligos from the plate will be counted on the nCounter platform or sequenced on an Illumina platform.

**Slide 3:**

Now, I will go into more details about the different strategies that the user can choose when he selects a region to profile.

In fact, this is the biological question that informs your region selection, so for example, if you want to assess the heterogeneity of expression in different regions of your tissue, you can place geometric shapes around specific areas of your tissue.

If you want to determine the expression profile of distinct tissue compartments, such as the tumour cells and its surrounding environment, you can choose to segment a ROI into more areas of interest (AOIs).

You can choose as well to draw concentric circles around a histological landmark, known as contour profiling, which is particularly useful to examine how the proximity to a biological feature affects the biological response.

You can grid across your entire tissue section as well to make a digital map of the expression profile of the tumour.

Finally, if you want to characterise expression changes within a specific cell type population, you may want to segment a specific cell type population.

**Slide 4:**

Okay so now, I will go into more details about the data analysis stage. If you remember well, after we retrieved all the DNA barcodes from the tissue section, they can either be counted on the nCounter platform or sequenced on the Illumina sequencer. But in any case, the user will be able to retrieve a matrix of counts, and is free to choose between a Data analysis suite embedded to the platform, and a programming language such as R, to process and analyse the data.

**Slide 5:**

But regardless of the method the user decides to use, it is essential to do a proper quality control and normalize the count data, to remove the technical variability in your data and to focus on the true biological signal of interest.

So, at first, you need to filter out segments or regions with very low signal strength. To that end, what you can do is to only keep the segments with a frequency (that you/the user is free to specify) of targets above an expression threshold.

Afterwards, you must filter out targets very low expressed across the different segments, by only keeping those with an expression above a threshold, at a specified frequency.

Now, you are ready to normalize the data, with four available strategies, but I will not go into as much detail since it is relatively tricky, but we can discuss this later if you’re curious to learn more about those.

**Slide 6:**

And now, you are ready to visualize and interpret your data. So, for example, if you want to visualize the distribution of protein of gene expression across multiple tissue compartments, you can generate some figures such as heatmaps or boxplots, while if you want to discover statistical changes in expression between experimental groups, you can create a volcano plot.

**Slide 6 to 9:**

So, here is an example of a heatmap that we can generate using the Data Analysis suite. Through this heatmap, we can visualize the distribution of expression of multiple protein targets across 4 large areas of the tissue, each composed of 3 “smaller” regions that the user selected to do the profiling. And we can clearly see, from this heatmap, that some proteins cluster together by their expression.

Another example of plot the user can generate is the bar chart, that is used to represent le proportion of different cell types across multiple brain compartments.

And last, you can make standard scatter plots, here used to compare the expression of the CD3 protein between 2 tissue slides, as well as boxplots.

**Slide 10:**

Few words now about the statistical tests you can employ to perform the differential expression analysis. So, assuming that the data is normally distributed, and that you have only two ROIs to compare, you will choose a standard t-test, which will be paired (if the ROIs come from the same slide) or unpaired (if they come from different slides).

But if your data is skewed, you will employ a Mann-Whitney U test if your two ROIs come from two different slides or a Wilcoxon signed-rank test if they come from the same slide.

Finally, if you need to compare gene or protein expression across more than two ROIs, which is the case most of the time, you will use linear mixed effect modeling, which corrects for batch effect.

**Slide 11 to 14:**

Finally, I will go through a few assets of the GeoMx DSP and a major limitation of its use.

So, first, as you might have understood before, the GeoMx works by directly counting mRNA or protein molecules, eliminating bias in the expression data as you might have with reverse transcription or PCR amplification in the sequencing process.

Secondly, it allows the user to get the profile, in the spatial context, of the whole transcriptome of human and mouse as well as up to 570 protein targets, separately or simultaneously.

You can process up to 10 slides per day, showing the speed of the technology.

The user is flexible to select the regions he wants to profile, based on the biological question he wants to answer.

In addition, when you analyze the data, the interface of the Data Analysis suite provides you with both the profiling and imaging data, and this is outlined in the following slides. As you can see here, if you move the cursor to the heatmap, it shows you straight where the count comes from on your tissue section.

Finally, the platform has many biological applications, you can use it for example to find biomarkers predictive of treatment response, to profile cancer cells and its tumor microenvironment (such as stromal cells and immune cells) or to reveal new molecular subtypes for diseases.

But, similarly to the Visium technology from 10X Genomics, the GeoMx is not conductive to single-cell analysis, you need at least 20 cells in a region you selected to get a good quantification of transcripts and proteins. And, in fact, to overcome this drawback, the CosMx SMI was designed, but its throughput is limited, since it enables quantification of up to 1000 mRNA molecules and 100 proteins.

**Slides 15 to 17:**

Okay, so before we end this journal club, I will give you a practical example of the use of this technology in the context of the biomarker discovery, through this following paper: **Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma (Amaria et al., 2018)**.

So, to contextualise, the current standard of care for patients with clinical stage III melanoma is upfront surgery with an adjuvant therapy, however, preclinical studies suggest that neoadjuvant ICB is associated with improved survival and enhanced anti-tumor immune response compared to the same treatment administered in the adjuvant setting.

But, so far, we were unable to validate this observation, since there were no clinical studies done to assess the effect of the neoadjuvant ICB in melanoma.

So, this is in this context, that Rodabe Amaria and her team conducted the first randomised neoadjuvant trial in patients with stage III and IV melanoma.

So this trial included 23 patients, where 12 were randomised to nivolumab and the others to nivolumab+ipilimumab.

And to better characterise the tumour molecular and immune microenvironment of these patients before and after treatment, they took tumour biopsies from them prior and after therapy, and passed them onto the GeoMx system to do the profiling.

Now the results, so at first, they showed that the combination therapy was associated with improved survival over the monotherapy.

But more importantly, in each treatment group, they noted that some patients responded better than others, and interestingly, when they compared the immune/ tumour cell profiling between the responders and non-responders, they observed a greater expression of PD-L1 and of some immune markers (Granzyme B, CD4, FoxP3, CD20, and PD-1), as well as higher CD8+ T cell infiltrate, in responders over the non-responders, both at baseline and early-on treatment.

And these observations are illustrated in the following plots. So in these two bar plots **b** and **c**, we can clearly observe a greater density of CD8+ T cells and a higher expression of PD-L1 in responders compared with the non-responders.

In the heatmap **d,** in which they summarised the expression of these immune markers in responders versus non-responders, both at baseline and early-on treatment, we can clearly see the signal for these immune markers is much higher in responders and non-responders.

And finally, this volcano plot is in support of these findings, since all the immune markers quantified by the GeoMx are significantly more expressed in responders than in non-responders, prior and after treatment.

So, now, to conclude, thanks to the GeoMx, we found markers that could be used to predict the success of the neoadjuvant immunotherapy in patients with high-risk resectable melanoma.

However, we need to be cautious in the interpretation of our findings, since this study has several limitations, including relatively small sample size, an early closure of the trial as 2 patients in the monotherapy group had disease progression preventing surgery and 8 patients that received the combination, had severe adverse events.

Finally, the inclusion of patients with metastatic stage IV melanoma, makes the study population heterogeneous and therefore decreases the generalizability of the results to a stage III population.

And, these data are limited to patients with cutaneous melanoma subtype, so we do not know if we can really generalise these findings to other subtypes, such as mucosal or acral subtypes.

**Drafts:**

**Slide 2:**

So originally, the GeoMx DSP was designed to profile gene or protein expression from bulk tissue samples, in a spatial context.

But you might wonder here, how is it possible to retrieve quantitative profiling data from a single tissue biopsy ?

And, in fact, there are 5 steps required to achieve. At first, onto a single tissue section, whether it is FFPE or fresh frozen, we combine two types of reagents together, the imaging reagents used to image or stain your tissue, and the profiling reagents used to profile it . So, essentially, the imaging reagents are fluorescent antibodies (for protein) or RNAscope probes (for RNA). And the profiling reagents are either antibodies (for protein detection) or ISH probes (for RNA), which are tagged with a DNA barcode specific to the protein or RNA molecule we target, via a UV-photocleavable linker.

So, once the sample preparation is done, the slide will go onto the GeoMx system, to be scanned, and will give a high-quality fluorescent image of up to 4 colours.

And, using the fluorescence of that image, this is the user that will select the regions of interest he wants to profile. Here for example, he decided to draw a geometric circle around a specific area of the tissue he wants to profile, but there are actually different available strategies the user can choose and I’ll come back to that later.

Subsequently, after the selection process is done, every region of interest will be sequentially exposed to UV light. And this exposure will cleave the linkers, and in turn, release the DNA barcodes, that will be aspirated by a microcapillary tube embedded to the platform, and dispensed into a 96-well plate.

**Final slide:**

And now, I will show you a case study of the use of the GeoMx DSP to determine biomarkers predictive of the response to immunotherapy.

So, in this article, Jennifer Wargo and her team at MD Anderson Cancer Center compared neoadjuvant monotherapy using nivolumab (anti PD-1) only to combination therapy using ipilimumab plus nivolumab (anti PD-1) + ipilimumab (anti CTLA-4). And they demonstrated two things, first that neoadjuvant treatment with combination therapy was associated with improved overall survival, over treatment with nivolumab alone. And especially, in both therapy groups, they noticed that some patients responded better than others. But how come? So to answer that, they used the GeoMx DSP to characterise the tumour microenvironment of responders and non-responders using tumour biopsies taken prior and early on-treatment. Technically speaking, for each tumour sample, they selected the hot immune regions and the tumour cells via a segmentation method and using the 2 morphological markers (S100B for tumour) and (CD45 for immune cells).

And, interestingly, in each treatment group, the profiling demonstrated higher CD8+ T-cell infiltrate, tumour cell PD-L1 expression, and expression of lymphoid markers (Granzyme B, CD4, FoxP3, CD20, and PD-1) in responders compared to non-responders. And interestingly, these differences were observed not early during treatment, but at baseline, which suggests that these markers might be used to predict the success of the neoadjuvant immunotherapy in stage III or oligometastatic stage IV melanoma patients.