Welcome to **CellCarta China Updates** – CellCarta’s scientific and operational bulletin. We created this medium to provide our clients with updates on our rapidly growing laboratory in Jining, China. To subscribe, please [contact us](#).

Thank you for the support and positive feedback on our previous issues of this newsletter. It is our privilege to share our scientific updates with you and learn how we can best support your clinical trial needs.

We are pleased to provide some exciting biomarker updates for our laboratory in China, including the addition of high dimension flow cytometry and information on Claudin 18 as a biomarker, which are relevant to many clinical trials in China.

We appreciate hearing from you. Your feedback guides our newsletter content, so we would love to hear what topics are of interest to you and how we can support your clinical trials.

“Thanks to you and your team for your strong support, especially your active help in coordinating and communicating in many unexpected situations. On behalf of our project team, I would like to express my sincerest gratitude. This project is progressing very well now, and the indications have been approved.”

– Pharma Client

**FLOW CYTOMETRY**

CellCarta offers [multiparametric cellular analysis](#) with off-the-shelf and customized flow cytometry panels. Our flow cytometry services in China are comprehensively supported with local project and HGRAC management teams. Our expert team in Montreal will perform the data analyses. Our laboratory in Jining is well-equipped to support testing on PBMCs (Image 1). As required, we can accommodate fresh and frozen samples.

Our Jining laboratory offers two platforms for your flow cytometry needs. The BD LSRIIFortessa™, a high dimensional flow cytometry platform, accommodates up to 18 colors (Image 2). We use this platform to provide a comprehensive characterization of cellular subsets which greatly informs drug development analyses and often supports the selection of patients for precision medicines.

We begin sample analyses on the Fortessa in Q4 2022. Clients familiar with our assays in North America and Europe can look forward to our panels being available in Jining with the same level of quality and precision. Please [contact our scientific team](#) to initiate discussions of your panels of interest.
Our BD FACSLyric™ is ideal for your clinical or regulatory applications. The Lyric™ is capable of running up to 12 colors, making it an excellent choice for targeted panels that may require primary or secondary endpoints for clinical or regulatory applications (Image 3). Our Jining laboratory offers a validated TBNK assay that can be used for monitoring lymphocyte percentages and/or absolute counts in your clinical trials, with over 30-day stability in whole blood samples. Our TBNK assay can also be used for tracking lympho-depletion in CAR-T studies and has been validated for secondary endpoint intended usage. Contact us to discuss your interests or customization of panels for your clinical trials.

Image 2. The BD LSRIIFortessa™ at our Jining laboratory. It accommodates up to 18 colors with globally standardized quality and precision parameters.

Image 3. Our Jining staff review data from the BD FACSLyric™, an ideal flow cytometry platform for your clinical or regulatory applications.

CLAUDIN 18 IHC AT CELLCARTA:
Acquire Target Information for Your Targeted Therapies

Claudin 18.2 (CLDN18.2) has become a popular target for antitumor drugs. CLDN18.2 targeted therapies under investigation include monoclonal antibodies, antibody-drug conjugates, bi- and tri-specific monoclonal antibodies, and CAR-T cell therapy. CellCarta can provide biomarker support for each modality in various tumor indications.

Currently, there are dozens of Phase 1 through Phase 3 clinical trials planned or in progress, with many of these clinical trials in China, including Shanghai, Beijing, Shenzhen, Chengdu, Zibo, Harbin, and Guangzhou. While gastric cancer and gastroesophageal junction adenocarcinoma predominate in clinical trials, pancreatic cancer, biliary tract neoplasms, and also CLDN18.2 positive mucinous ovarian are among the cancers being evaluated. Most of these clinical trials require evidence of CLDN18.2 protein expression in these neoplastic tissues by immunohistochemistry (IHC). CellCarta IHC assay development scientists have evaluated ten commercially available CLDN18 antibodies (see Table 1), and CellCarta has developed and fully validated CLDN18 IHC.

Table 1. Commercially Available CLDN18 Antibodies Tested by CellCarta

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Catalog Number</th>
<th>Clone</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abcam</td>
<td>ab203563</td>
<td>34H14L15</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Abcam</td>
<td>ab222513</td>
<td>EPR19203</td>
<td>Rabbit IgG</td>
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<tr>
<td>Abcam</td>
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<td>EPR19202</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Creative Biolabs</td>
<td>HPA-B1020-Y1</td>
<td>43-14A</td>
<td>Mouse IgGx</td>
</tr>
<tr>
<td>Invitrogen</td>
<td>700178</td>
<td>34H14L15</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>LSBio</td>
<td>LS-B16145</td>
<td>LS-B16145</td>
<td>Mouse IgG</td>
</tr>
<tr>
<td>LSBio</td>
<td>LS-B940</td>
<td>Polyclonal</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Novus Bio</td>
<td>NBP2-32002</td>
<td>Polyclonal</td>
<td>Rabbit IgG</td>
</tr>
<tr>
<td>Proteintech</td>
<td>66167-1-IG</td>
<td>5G7F2</td>
<td>Mouse IgG2a</td>
</tr>
<tr>
<td>Ventana (Roche)</td>
<td>790-7027</td>
<td>43-14A</td>
<td>Mouse IgGx</td>
</tr>
</tbody>
</table>

FLOW CYTOMETRY (continued)
The Claudin proteins are transmembrane tight junction proteins, meaning they control the movement of water and other substances between cells. CLDN18 has two isoforms, simply called 18.1 and 18.2. With the exception of gastric epithelium, evidence suggests CLDN18.2 is not expressed in normal healthy tissues. Furthermore, the conformation of CLDN18.2 in healthy stomach is such that antibodies do not appear to bind to the protein in vivo, suggesting potential for lower drug toxicity. In tumors, CLDN18.2 is thought to be more accessible because it is “out of place,” or ectopically overexpressed. This also contributes to its popularity in the targeted therapy space.

CLDN18.1 and 18.2 are similar in that they are both 261 amino acids; they differ only in the N-terminus. As such, target specificity is both a challenge for drug developers and an important consideration when developing IHC methods. When validating an immunohistochemistry method, it is important to evaluate specificity. Cell lines and orthogonal methods such as Western blot or qPCR are sometimes used at CellCarta, especially when similar isoforms exist, and differentiation is needed. The CLDN18.1 isoform is predominantly expressed in lung, while 18.2 is contained within normal gastric mucosa epithelial tight junctions. Normal and tumor tissues are evaluated and compared to the expected staining patterns routinely as part of IHC validation (Images 4 and 5).

In their 2022 *Chinese Medical Journal* article, Xia et. al emphasize that cancer is the leading cause of death in China and developed countries. They estimated there will be ~509,421 new cases of stomach cancer in China in 2022 (compared to ~27,294 in the USA). The same study estimates ~400,415 deaths in China this year, versus ~11,898 in the USA. While they showed that rates of stomach, liver, and esophageal cancer decreased gradually in China, treatments are still desperately needed. There are several antibodies being used in clinical trials for IHC, with varying cut-offs for patient inclusion. CLDN18.2 targeted drugs require further evaluation in clinical trials and will no doubt continue to include evaluation of tumors by IHC. Please contact us to discuss your IHC assay development and validation needs, or to include one of our validated IHC assays in your clinical trials.
CLAUDIN 18

- Two very similar isoforms (18.1 & 18.2)
  - Isoforms differ only in the N-terminus of the protein
  - 18.2 is exclusively and highly expressed in healthy stomach
  - 18.2 expression is seen in a subset of gastric cancers
  - 18.2 expression levels in tumors can range from negative to strongly positive
  - 18.2 is a popular target for anti-tumor drugs
- CLDN18 is evaluated by immunohistochemistry (IHC) in clinical trials
- Positivity by IHC is an enrollment criterion for many CLDN18.2 targeting drugs
- CellCarta has evaluated ten CLDN18 antibodies by IHC
- CellCarta employs robust IHC validation procedures for IHC deployment in all clinical trials phases

References & Additional Reading:

1. Claudin18.2 is a novel molecular biomarker for tumor-targeted immunotherapy | Biomarker Research | Full Text (biomedcentral.com)
3. Frontiers | Clinicopathologic Relevance of Claudin 18.2 Expression in Gastric Cancer: A Meta-Analysis (frontiersin.org)
4. Evaluation and reflection on claudin 18.2 targeting therapy in advanced gastric cancer - PubMed (nih.gov)

If you missed earlier issues of this newsletter and would like a copy, or if you would like to receive these monthly updates, please contact us. We will continue to use this newsletter to keep you informed of our scientific updates and services to assist you in the planning of your clinical trials in China. We thank you for allowing us to be your global partner of choice for precision medicine solutions.