Due to a lack of robust *in vivo* gut models, animal studies are generally required to evaluate gut toxicity. However, these studies can be lengthy and expensive and may not accurately recapitulate the behavior of the human gastrointestinal tract. Drugs are thus often developed with undesired gut side effects that are not apparent until clinical trials.

INCREASING THE EFFICIENCY OF DRUG DEVELOPMENT WITH PRECLINICAL TESTING USING HUMAN INTESTINAL STEM CELLS

By Ron Laethem, Altis Biosystems

ALTIS BIOSYSTEMS

Organ-on-a-chip models are the closest *in vitro* options, but they still have many limitations. More efficient development of drugs with attractive side effect profiles requires robust, easy-to-use, cost-effective *in vitro* gut models that are physiologically representative of the human intestinal tract and thus predictive of *in vivo* behavior. RepliGut® tissue constructs have been designed to meet this need.

Lack Of Robust *In Vitro* Gut Models

Very few *in vitro* models available accurately represent the gut. Some immortalized or cancerous cell lines, such as Caco-2 cells, are relatively simple to use and enable studies to be conducted fairly quickly. But the translatability of the data from *in vitro* to *in vivo* is always a question, especially since these are cancerous rather than normal cells. For studies of gut toxicity, animals are needed, and development timelines increase significantly when transitioning from the petri dish to *in vivo* evaluation. The higher up the evolutionary scale the model animal being used, the more that cost and timelines increase as well — it is much more expensive to conduct studies in non-human primates than in rats or mice.

The lack of robust *in vitro* gut models can be attributed to the complexity of gut biology, which makes it difficult to recapitulate. Even in animals relatively close to humans — such as dogs, a fairly popular species for tox studies — the gastrointestinal tract reacts quite differently than it does in humans. For instance, dogs are more prone to emesis (vomiting) and nausea. As a result, the translatability is questionable. While nonhuman primates are fairly good models for people, they are very expensive, and costs can skyrocket with these studies.

Intestinal organ-on-a-chip models are probably the closest *in vitro* options that exist today. While a few are commercially available, each has issues. Some are very expensive and require specialized equipment to implement. Throughput is typically the greatest limitation, and there are often challenges associated with sampling and analysis. The accuracy of predictions obtained using these models can also vary widely.

There is consequently a need for more robust, easy-to-use, cost-effective *in vitro* models that are physiologically represen-tative of the human intestinal tract and thus predictive of *in vivo* behavior.

The Example Of Gut Side Effects

To illustrate the difficulties, one good example is the potential for gut side effects caused by chemotherapeutic drug candidates. There are currently no robust *in vitro* models for use in frontline screening of compounds for potential gut side effects during discovery and lead optimization. Consequently, *in vitro* testing is employed to answer a host of questions about the properties of the candidates and how they will behave *in vivo*, but this issue is ignored.

In some cases, animal studies may be performed, but they may not be predictive regarding gut toxicities. As a result, the first indication that gastrointestinal adverse events (e.g., diarrhea, constipation, intestinal distress) could be a problem appears during human clinical trials. By that time, it is late in the development process, and it is not typically possible to go back and redesign the molecule to eliminate the issues and improve

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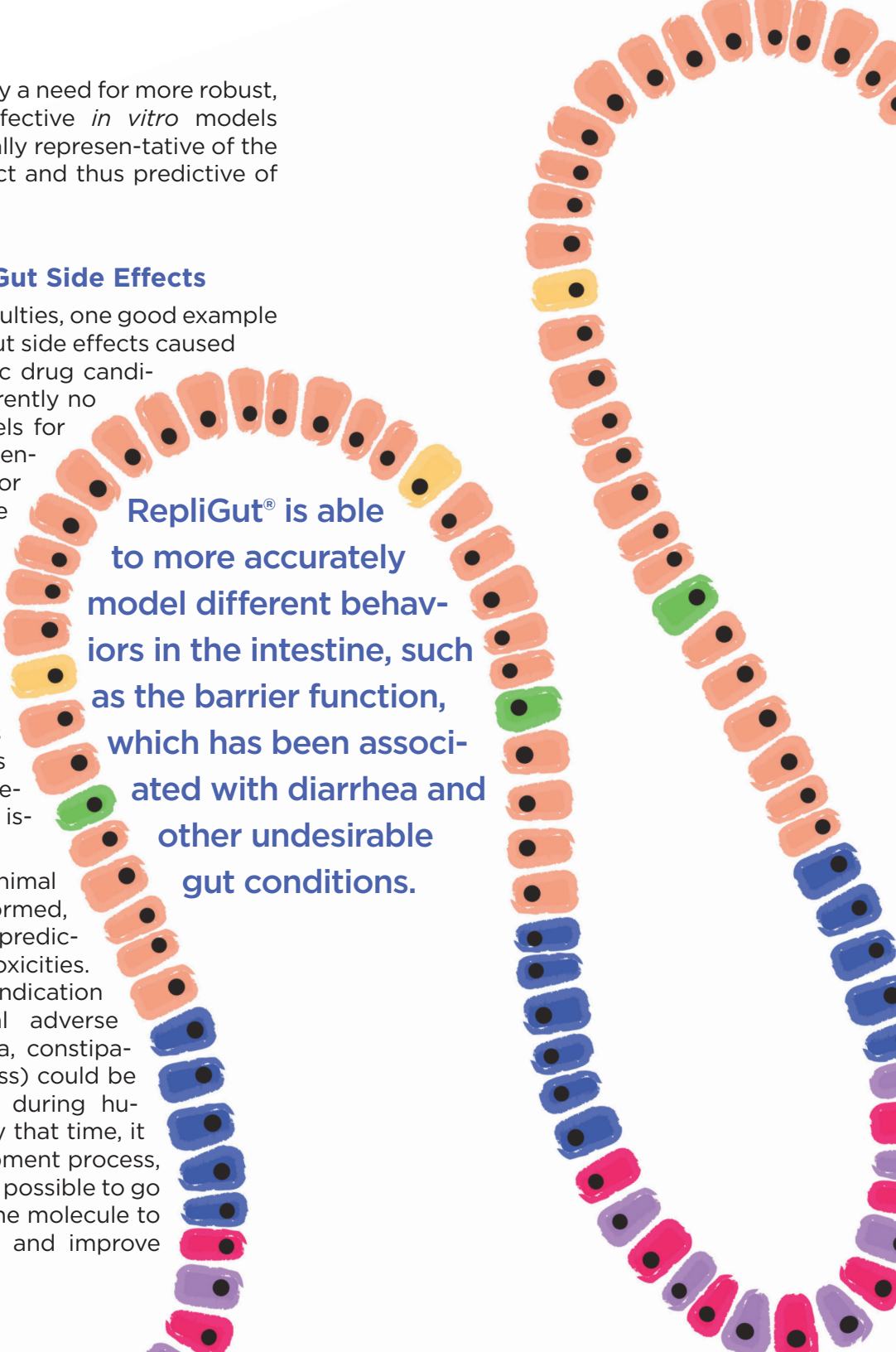

the side effect profile. This gastrointestinal distress often results in compliance issues that can compromise the benefits of therapy. The general solution is to prescribe palliative cotherapies to resolve the issue.

If potential gut toxicities could be explored using *in vitro* studies during the preclinical development stage, it would be possible to eliminate candidates with this liability early in the project timeline, saving both time and money. Clearly, fewer liabilities carried into the clinic will result in higher-quality drugs that reach the market more quickly.

An Efficient Human Cell–Based Option

RepliGut® tissue constructs from Altis Biosystems have the potential to address this critical need in pharmaceutical drug development. Based on human intestinal stem cells, RepliGut® eliminates the issues associated with the use of colon cancer cells. The polarized monolayers express tight junction proteins and recapitulate the barrier function of the intestine *in vivo*. Additionally, they can be tailored to consist of different cell lineages. Because they are healthy, normal (not cancerous) human cells, they provide more physiologically relevant results. Intestinal stem cells used for the RepliGut® system are harvested from transplant-grade human donor tissue for which there is no recipient match. As such, the stem cells are very high-quality with very low necrotic or ischemic damage.

Each tissue sample in a RepliGut® kit features a biomimetic scaffold that separates RepliGut® cells from the cassette's porous membrane and allows RepliGut® cells to survive for a prolonged period of time. Luminal and basal reservoirs allow compounds and additional cell types to interact with the epithelial cells for side-specific assays.

With this design, RepliGut® is able to more accurately model different behaviors in the intestine, such as the barrier function, which has been associated with diarrhea and other undesirable gut conditions. In some cases, it may be possible to eliminate the need for animal studies. The potential for efficiency gains and reductions in time and cost are therefore significant.

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Developing Data Sets

Achieving a high level of adoption of new assays, such as those based on the RepliGut® system, is challenging, even when the potential benefits are measurable. It is even harder when the methods being re-placed are as well-entrenched as Caco-2–based analyses.

Typically, it takes the interest of a leading pharmaceutical company to drive movement within regulatory agencies. If a big pharma company takes data based on a new method to the U.S. Food and Drug Administration and shows the agency that the method is robust and reliable, the regulators will usually pick up the baton and investigate further. Small companies like Altis, even with tremendous positive data, typically cannot do it alone; they definitely need a buy-in from big pharma. Luckily, Altis works with half of the top-20 pharma companies and hopes to work with more in the future.

We are currently focused on developing extensive data for specific functionalities of the RepliGut® system. There are many potential applications, and we are taking a stepwise approach to test each application. Because the barrier function of the intestine is its key feature, we have initially focused on the development of a RepliGut® system useful in related assays. This system currently focuses on epithelial cells, and RepliGut® offers a really robust barrier function on par with Caco-2 cells.

Altis has used RepliGut® to test a number of compounds in collaboration with AstraZeneca, but we need to conduct more in-depth studies and compare results obtained with RepliGut® to those obtained in the clinic to establish with confidence that the system accurately mimics what occurs *in vivo*.

Looking forward, there are many other aspects to intestinal biology that we could potentially assess with RepliGut®, and we will be taking a similar approach to determine if there is a robust correlation between the *in vitro* and *in vivo* data.

Potential Applications For RepliGut®

RepliGut® has the potential for many applications, including screening of completely novel compounds with new modes of action or identification of derivatives that operate by known mechanisms but have the potential to offer benefits over currently marketed drugs.

In the latter case, new drugs that have the same mechanism of action but no gut toxicity that can lead to unwanted side effects like diarrhea would have a huge advantage and likely displace existing products on the market with those side effects. Afatanib is one example — the clinical incidence of serious diarrhea with this drug is approximately 96%, and it disrupted barrier function with Repli-

Gut®. A new drug lacking that liability or with a reduced liability would be very attractive.

In addition to the cost savings achieved by eliminating non-representative *in vitro* and expensive, time-consuming and potentially non-correlative *in vivo* animal studies, the use of RepliGut® in gut toxicity assays has the potential to add immeasurable value in the form of improved health and quality of life. Both the patients and the drug manufacturer would benefit.

Elimination of animal testing also has a societal benefit. There is a strong push in the pharmaceutical industry to reduce and if possible eliminate animal testing for ethical reasons. There is as a result a desire to recapitulate *in vivo* tests conducted with animals using *in vitro* and in silico models that have been proven to provide robust correlations.

Greater Life Span Will Further Boost Efficiencies

There are many opportunities to expand the functionality of the RepliGut® platform, and Altis is working on many of these. Currently, most RepliGut® assays are fairly short-lived; after the stem cells are differentiated, there are no more dividing cells and the cells are all terminal. The lifespan of these mature, differentiated cells in the gut is only five to seven days. In our model, therefore, after about seven days of differentiation, the cells start to slough off just like they would *in vivo*. Since there are no stem cells to replace them, and the cultures die off.

In the human intestine, however, there is a mix of stem cells and differentiated cells, with cells present at different stages of maturity. The stem cells continue to divide and reproduce and then differentiate, replacing the ones that slough off.

Altis is working on models in which both proliferating stem cells and differentiated cells are present in the same well to length-en the lifespan of our RepliGut® tissues and enable testing of drug effects on the entire system, rather than just differentiated cells.

In one of our prototype systems, we have maintained tissues for 30 days. We would like to extend that even further, so more detailed studies can be performed using an even more physiologically relevant model. This type of long-lasting model also presents the best opportunity to eliminate animal testing and increasing testing efficiency, because the system would be more complete and more reflective of the physiological gut.

Driving Adoption Through Partnering

In addition to driving the interest of regulatory agencies, partnering with big pharma companies helps to build credibility in the eyes of other drug development companies of novel assays, such as those based on the RepliGut® platform. Large pharma firms tend not to institute or implement new methods that don't provide high-quality, reliable data. Attracting the attention of big pharma first requires extensive research and testing to demonstrate the robust performance and the method.

Altis has already done much internal work and will continue to pursue R&D programs designed to further build out our body of evidence supporting the use of the RepliGut® platform for drug screening and other applications. We will also continue to expand our existing collaborations with big pharma companies and actively publish and present the results of these studies. The focus is to make the models better for them with respect to both ease of use and performance.

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