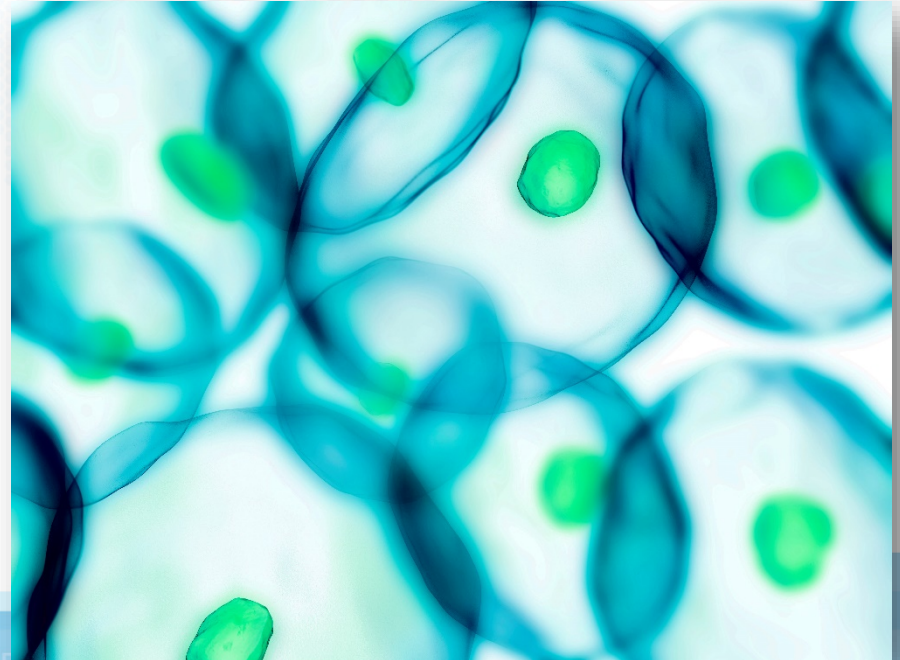


PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE
FROM DISCOVERY THROUGH CLINICAL SUPPORT

Which Hepatocyte Test System Should I Use? Supporting Preclinical DMPK

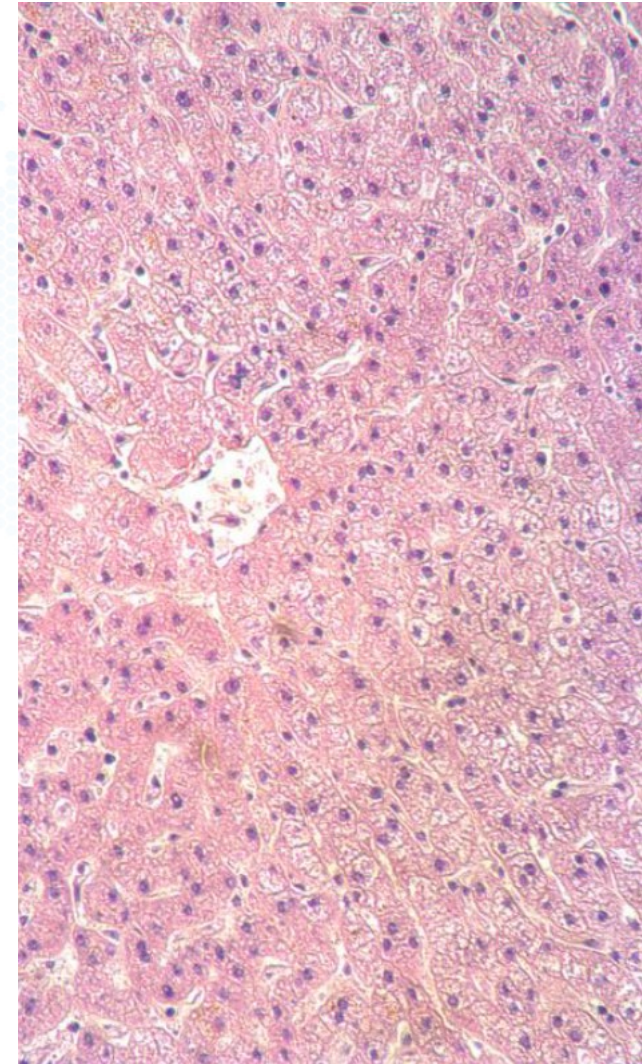
Chris Bohl

Global Technical Support - Products
cbohl@xenotechllc.com



Hepatocytes

- Hepatocytes are the main structural and functional parenchymal cells of the liver and they make up approx. 80% of the volume, but only approx. 60% of the cells.
- The liver has multiple functions, many which are indispensable for biological homeostasis at the organism level.
- Isolated hepatocytes can be useful models for many disciplines, but they are commonly used by ADME/DMPK scientists to predict in vivo drug metabolism and stability.



Why use Hepatocytes instead of hepatic subcellular fractions?

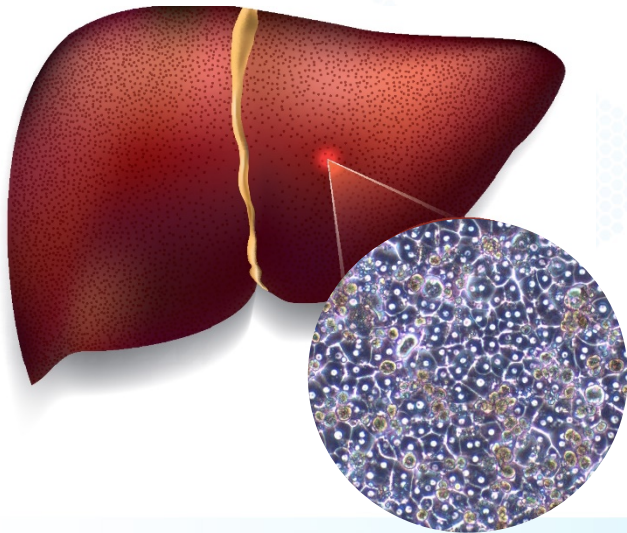
- They are the “gold standard” for ADME/DMPK
 - Living test system
 - Dynamic biochemical pathways in addition to the xenobiotic metabolic activities in hepatic subcell fractions
 - They are more representative

But...

- Individual donors can be limiting in quantity
- Need more refined techniques for reproducibility
- Higher costs

There are so many choices of test systems... which should I use?

Not all are appropriate for all assays!



Primary vs Immortalized Hepatocytes

Primary Hepatocytes – isolated from living tissue, genetically un-modified, terminally differentiated, limited time in culture.

Immortalized/Transformed Hepatocytes – spontaneous or purposeful genetic modification(s) that uncouple the biochemical pathways that control typical growth characteristics (senescence, proliferation, death, etc.)

Fresh vs Cryopreserved Primary Hepatocytes

Fresh Hepatocytes

- Never been frozen for storage.
- Offered in suspension or plated
- Accepted test system
- Need to be used immediately
- Will have some donor information, but won't know enzymatic activities that are commonly characterized in cryopreserved hepatocytes.

Cryopreserved Hepatocytes

- Carefully frozen to suspend cellular activity for long term storage.
- Convenience of being able to thaw and use cells at ones discretion.
- Long term stability
- Multiple formats
- Full enzymatic characterization
 - Post thaw viability, Phase I and Phase II activities, uptake, fold induction, optimal plating density, monolayer micrographs, and CL_{int} rates for select CYPs.
- Accepted test system

Animal vs Human

Primary Hepatocytes

- Primary animal hepatocytes can be used for species comparison to help choose the most relevant small animal model for *in vivo* work.
 - Cyno monkey, S-D rat, CD-1 mouse, Beagle dog, Gottingen Minipig, New Zealand white rabbit, Golden Syrian hamster, and Hartly albino guinea pig.
- Animal hepatocytes may have better availability compared to human.
- Can be a significant difference in costs.

But...

- Primary human hepatocytes are the most relevant
- Primary Human hepatocytes are the accepted test systems for certain assays required by the regulatory agencies (ex. CYP induction assays)

Which format?

- **Suspension cultures** – Hepatocytes that are not used while attached to a surface.
- **Plated cultures** – attach to collagen coated surfaces and form a confluent monolayer of hepatocytes (>85% coverage) for at least 5 days.
- **Individual donors** – all the cells are from the same individuals.
- **Pooled donors** – cells from multiple donors are mixed and used together.

Who Uses Hepatocytes?

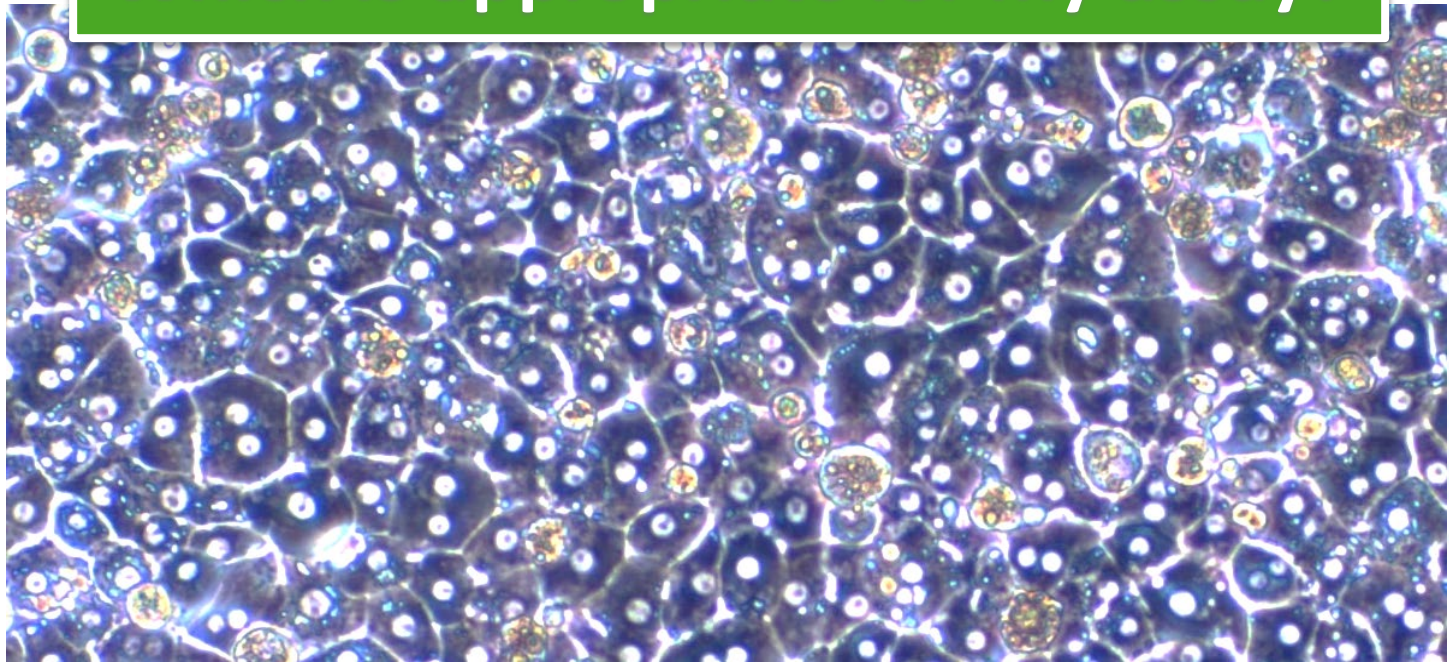
Screening/Discovery, R&D, ADME/DMPK groups, and Academia



Hepatocytes

There are so many choices of test systems...
which should I use?

Which is appropriate for my assay?



Primary or Immortalized

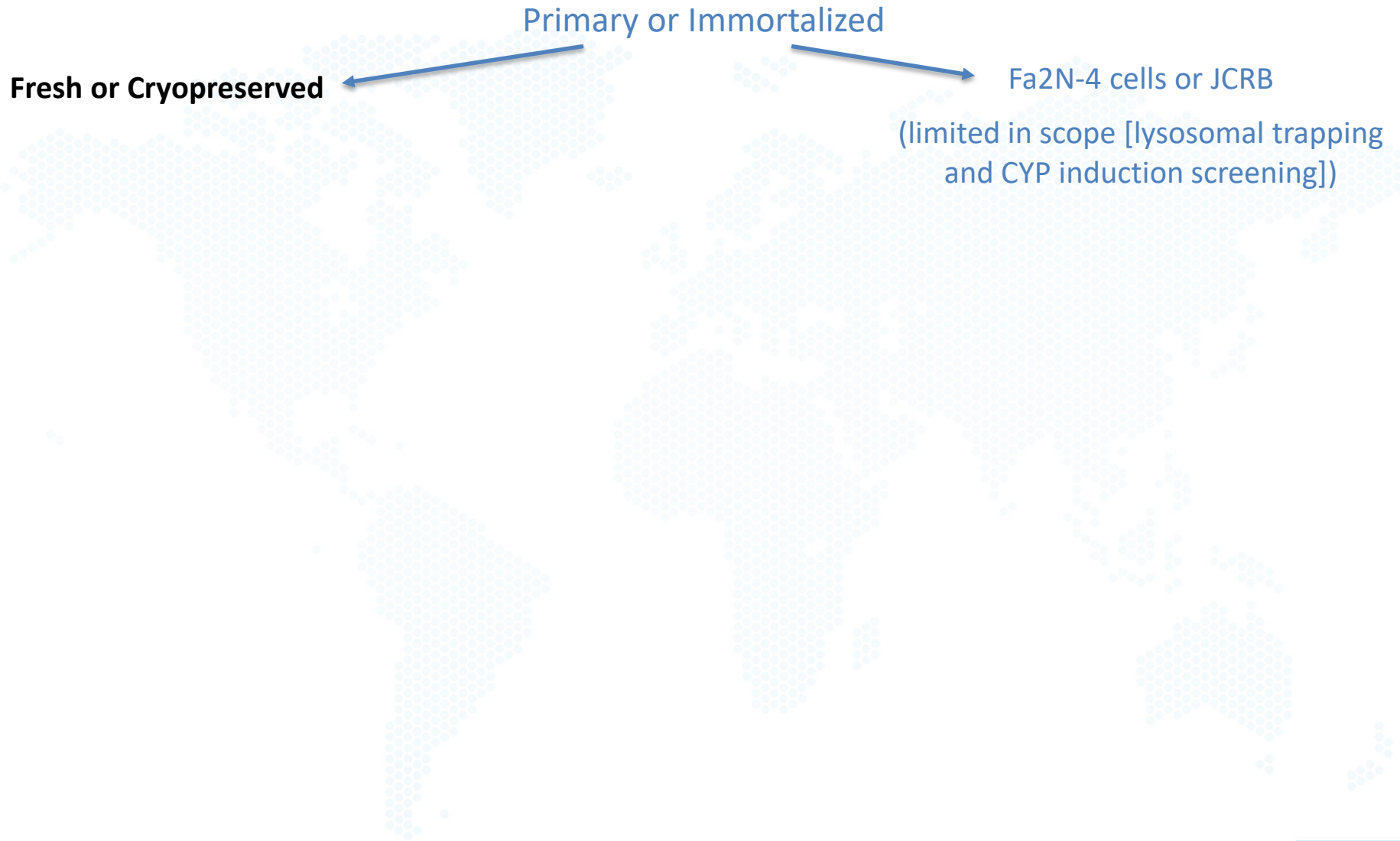


Primary or Immortalized



Fa2N-4 cells or JCRB

**(limited in scope [lysosomal trapping
and CYP induction screening])**



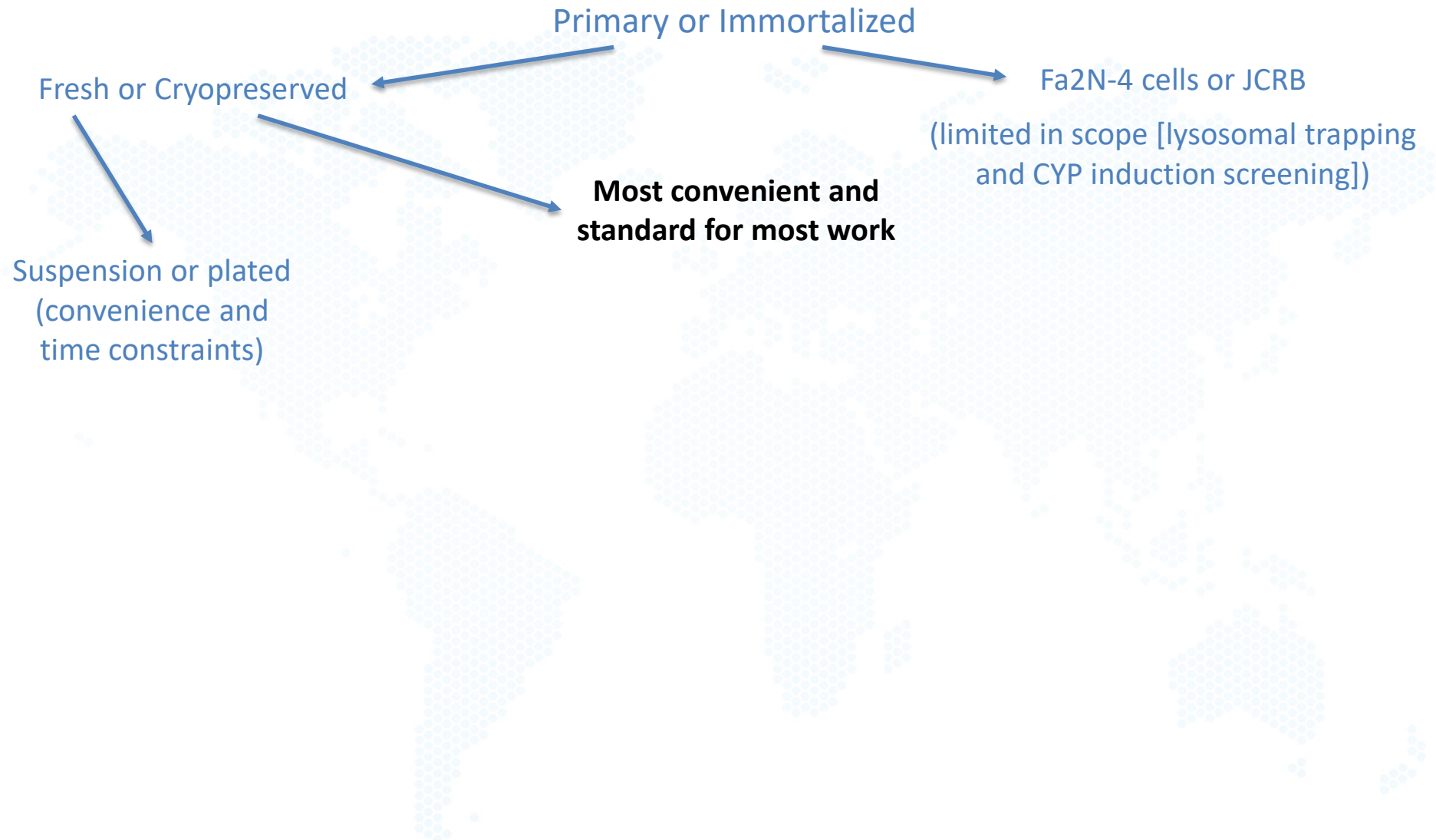
Primary or Immortalized

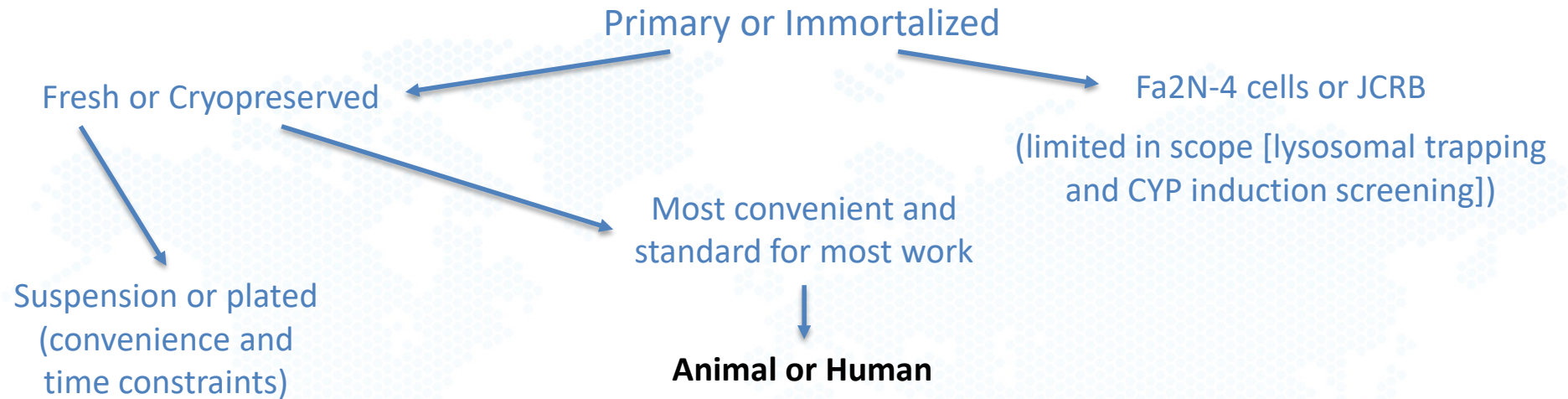
Fresh or Cryopreserved

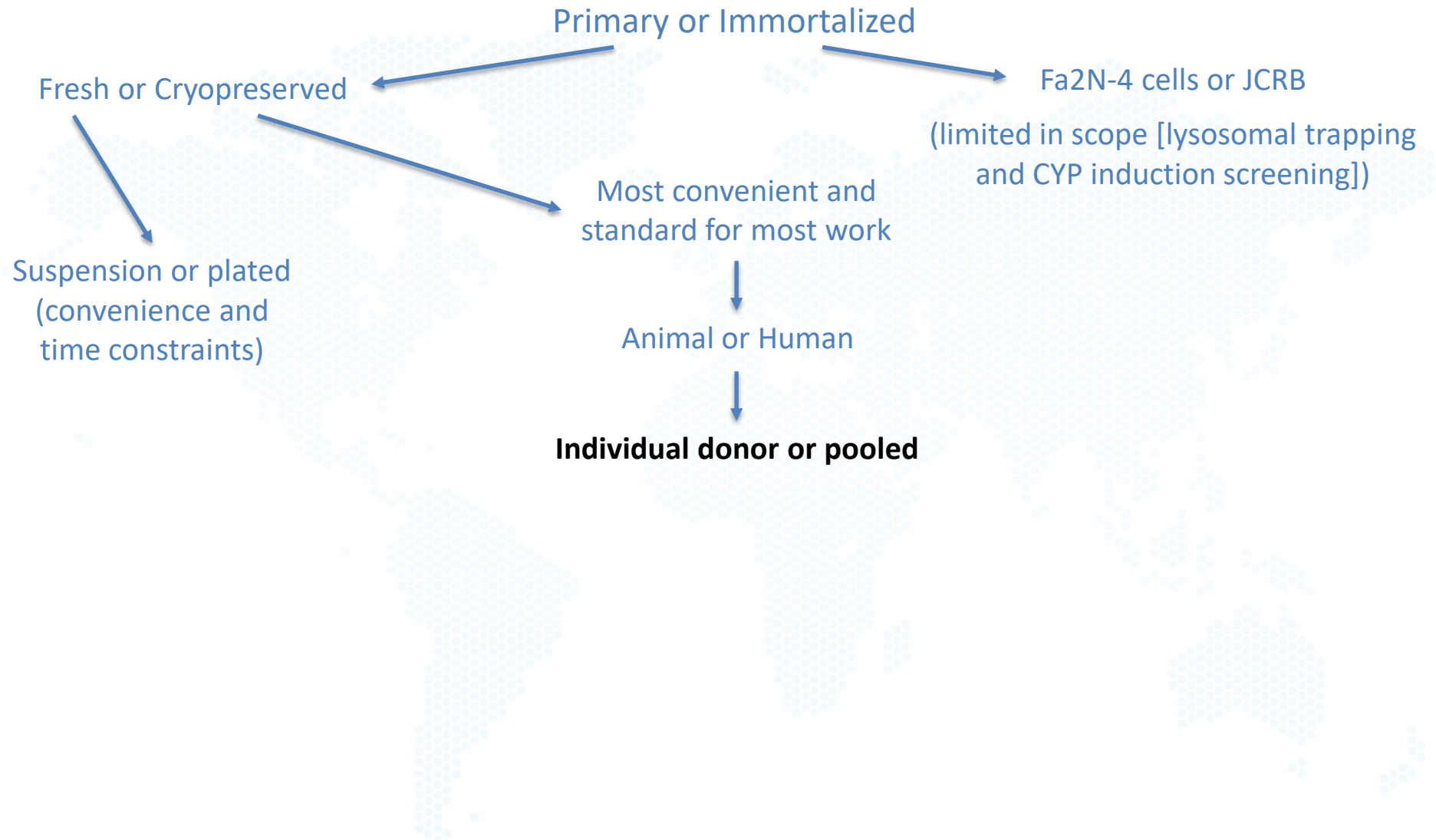
Fa2N-4 cells or JCRB

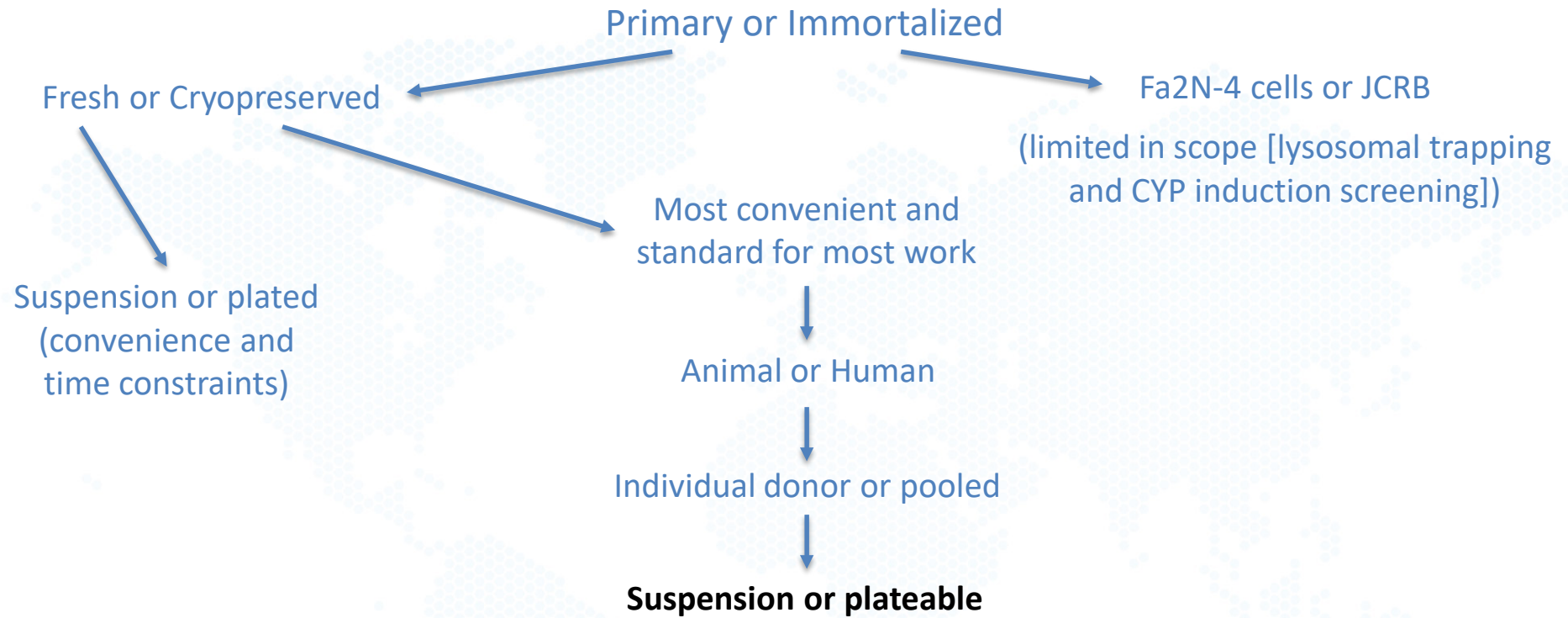
(limited in scope [lysosomal trapping
and CYP induction screening])

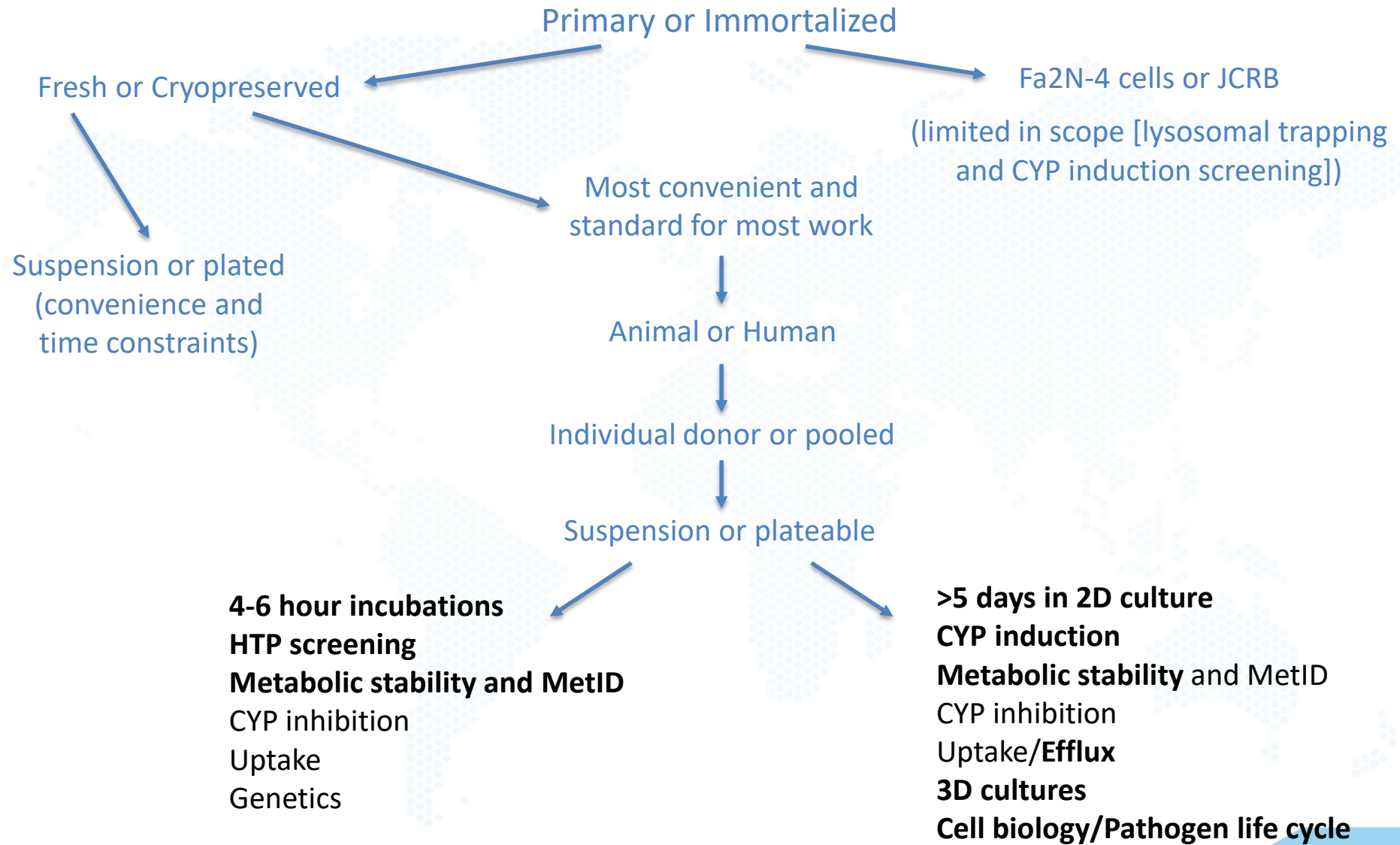
**Suspension or plated
(convenience and
time constraints)**











General Recommendations for Primary Hepatocytes

Suspension (4-6 hour incubation)

- > 70% post-thaw viability
 - Higher doesn't always mean better.
- Generally pools are preferred
 - Reduce donor dependent variability

Attaching (at least 5 days in culture)

- Post-thaw viability is less important because high viability doesn't always mean good monolayer.
- Good monolayer will cover >85% of the surface and last at least 5 days.
- Not all lots are recommended for plating in every well format.
- Induction assays
 - **>6 fold-mRNA induction**
 - **>2 fold-activity induction**
- Just because it forms a monolayer doesn't mean that it will be appropriate for your studies.



Customizable, single-freeze pooled
hepatocytes (n = 2 to 20)



Two-freeze hepatocyte
100 donor pool

Single Donor Human Hepatocytes

Single donor cryoplateable lots characterized
for CYP induction and/or uptake transporters
(CryostaX and traditional format)



Customizable, PLATEABLE single-freeze
pooled hepatocytes (n = 2 to 20)

Geneknown CryostaX Human Hepatocytes

Pooled genotyped human hepatocytes

<https://www.xenotech.com/webinar-videos/cryostax-pellets-improving-hepatocyte-performance>

RODENTS

HUMANS & OTHER ANIMALS


	Opti THAW <u>THAWING MEDIA FOR ALL ASSAYS</u>	Opti INCUBATE <u>MEDIA FOR SUSPENSION ONLY</u>	Opti PLATE <u>PLATING MEDIA</u>	Opti CULTURE <u>MONOLAYER MAINTENANCE</u>	Opti MATRIX <u>EXTRACELLULAR MATRIX</u>
RODENTS	K8100 for mouse or K8800 for CryostaX	K8400	K8200	K8300	K8600 or K8650
HUMANS & OTHER ANIMALS	K8000 or K8500 for HepatoSure	K8400	K8200	K8300	K8600 or K8650

Media Guide

Human and Animal Tissue Procurement

- Work with Organ Procurement Organizations for donated human tissue.
 - Non-transplantable tissue
 - Consent for research
 - Only obtain tissue from USA
- XenoTech has a strict set of criteria to ensure that livers are healthy and of the highest quality available.
- Negative serologies for major human pathogens, now including SARS-CoV-2.
- 24 hour on call team that can process the tissue the moment it arrives at our facility.
- All animals are procured through accredited vendors and are also free of major pathogens.
 - Animals are not housed on site.
 - Protocols are reviewed and IACUC approved
 - Have CITES for non-human primates

Thank you for watching!

Questions?

cbohl@xenotechllc.com

Get in touch through the **Contact Us** tab on our website

Call us (913) GET-P450

1-800-588-7530

