NeuroSAFE

Leverage Human Stem Cell Technology
&
Robotic Process Automation

By Dr. Subhadra Dravida

Clients & Partners

Top-Tier Academic & Commercial Partners

Safety (Neurovirulence) Concerns of Vaccines Historical

From 1955 to 1963, some batches of polio vaccine given to the public contained live polio virus, even though they had passed required safety testing –Resulted in many cases of **paralysis.**

In 1976 there was an increased risk of a **serious neurological disorder called Guillain-Barre Syndrome (GBS)** following vaccination with a swine flu vaccine

In 1998, some research caused concern that hepatitis B vaccination might be linked with **multiple sclerosis (MS),a progressive nerve disease**

There were concerns that the meningococcal vaccine Menactra caused a **Serious neurological disorder GBS**, Between 2005 and 2008,there were a number of youth who reported GBS after receiving Menactra

An increased risk of **narcolepsy** was following vaccination with Pandemrix, a monovalent 2009 H1N1 influenza vaccine in several European countries during the H1N1 influenza pandemic

Safety Concerns of Covid-19 (Neurotrophic) Vaccines
Scientific & Clinical Evidence
(In our life time)

Corona Viruses

Invasion into CNS: Olfactory epithelial cells, Cerebral endothelial cells, Nerve terminals of Vagus nerve in the respiratory and gastrointestinal tract

Human Coronaviruses infect the upper respiratory tract

In newborns, infants, immune-compromised individuals, the elderly infect the lower respiratory tract

Pneumonia, Asthma, Severe acute respiratory syndrome

Human corona viruses are neuroinvasive and neurotropic inducing an over activation of the immune system

Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study
June 25, 2020; THE LANCET

Neurological complications of coronavirus infection; a comparative review and lessons learned during the COVID-19 pandemic
J Neurol Sci. 2020 Oct 15
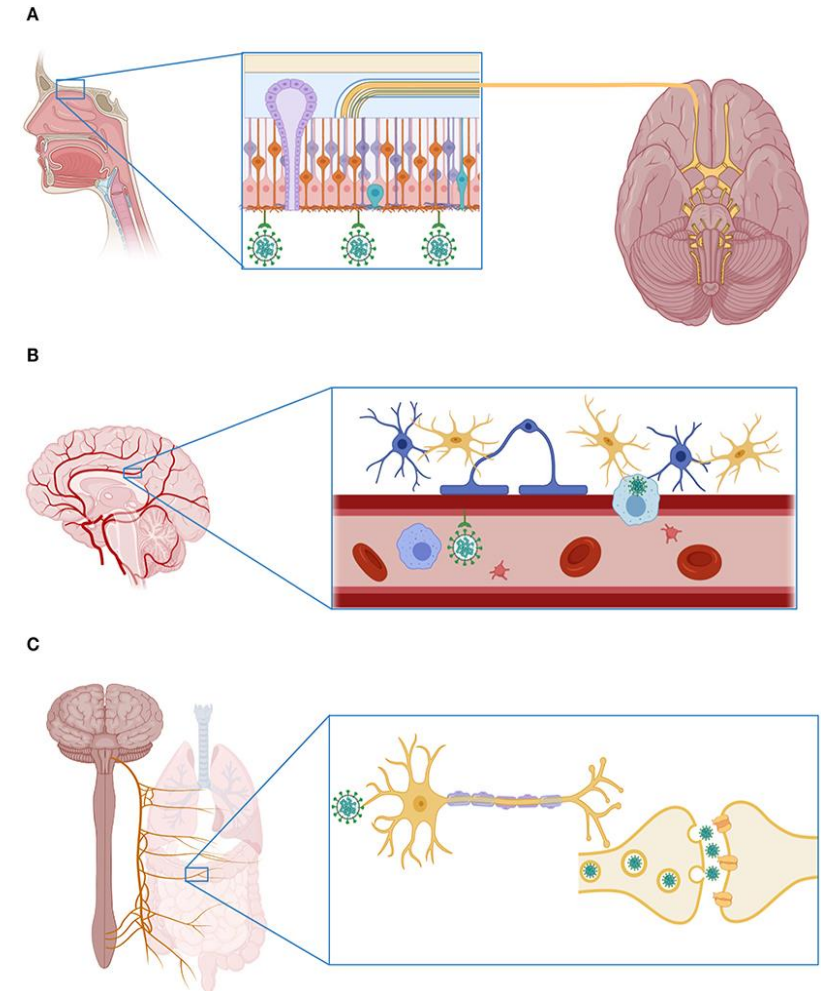
Front. Neurol., 04 September 2020

Safety Concerns of Covid-19 Vaccines
Real time Evidence

FDA attaches warning of rare nerve syndrome to Johnson & Johnson vaccine; NY Times July 2021

Japan: Black particles found in Moderna vaccine, batch put on hold Sep 2021

Drug Saf. 2021 Aug 4;1-6. doi: 10.1007/s40264-021-01104-9

Cumulative Adverse Event Reporting of Anaphylaxis After mRNA COVID-19 Vaccine (Pfizer-BioNTech) Injections in Japan: The First-Month Report

Myocarditis and Pericarditis After mRNA COVID-19 Vaccination

Functional Neurological Disorder After SARS-CoV-2 Vaccines: Two Case Reports and Discussion of Potential Public Health Implications July 2021

The Journal of
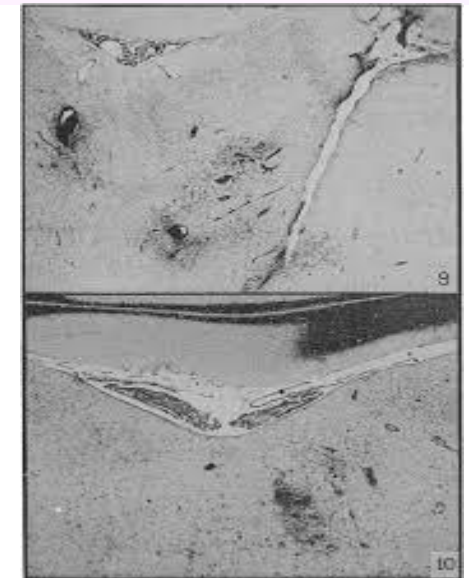Neuropsychiatry
and Clinical Neurosciences
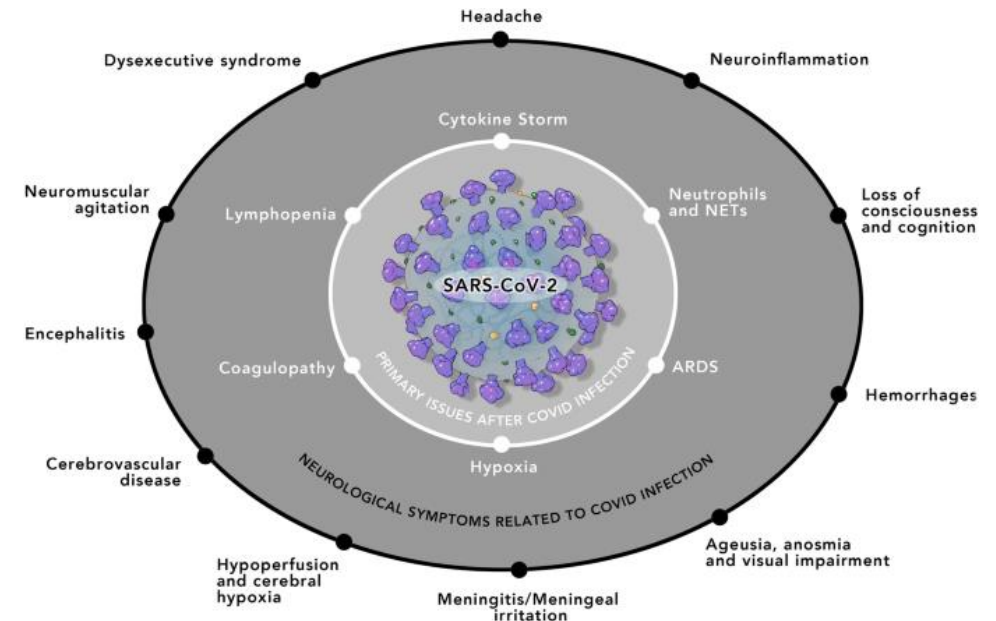
Testing Neurovirulence
Traditional – Gold Standard

For live, attenuated vaccines derived from neurotropic wild-type viruses, regulatory authorities require neurovirulence safety testing to assure the absence of residual neurotoxicity

MNVT

means

Monkey Neurovirulence Safety Test

iPSC Based In vitro Models
(Peer Reviewed Concept)

Merck Publication

Review

Live Viral Vaccine Neurovirulence Screening: Current and Future Models

Corey May Fulton * and Wendy J. Bailey

Safety Assessment and Laboratory Animal Resources, Merck & Co., Inc., West Point, PA 19486, USA;
wendy_bailey@merck.com
* Correspondence: corey.may.fulton@merck.com

5.2. Human Induced Pluripotent Stem Cells

Human induced pluripotent stem cells (hiPSCs) are an emerging technology that have been developed for multiple organs. To create hiPSCs, fibroblasts are harvested from patients, then induced to become stem cells, which can then be differentiated into human cells in culture. The stem cells can be maintained in culture precluding the need for repeated harvesting of stem cells. This process allows for the study of primary human cells without needing to collect them from patients and allows for larger scale studies as the populations can be grown in vitro. The opportunities for hiPSC-derived neural cell lines in studying virus-CNS interactions have been reviewed by Harschnitz and Studer [96]. Notable conclusions from that paper that should be considered are that the hiPSC models allow for scalability and flexibility in models; that they allow for high-throughput screening; and that they more closely mimic the immune responses of cells of the central nervous system than other models. All of these factors indicate that they would be potential models for use in determining the neurovirulence of live viral vaccines.

Transcell Oncologics

Pioneers in human stem cell tech based research for 15+ years –
technology platform for cancer patients (Hemato)
technology platform for the global pharma and biopharma (Drug discovery & Transtoxbio)

www.transtoxbio.com
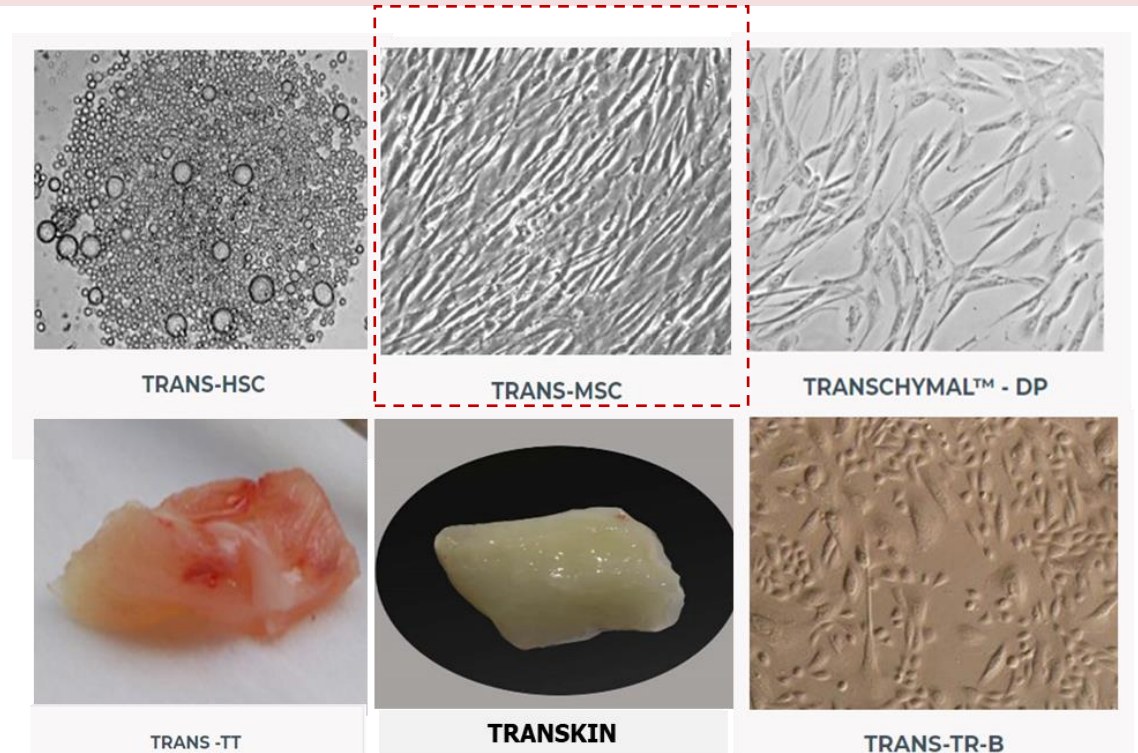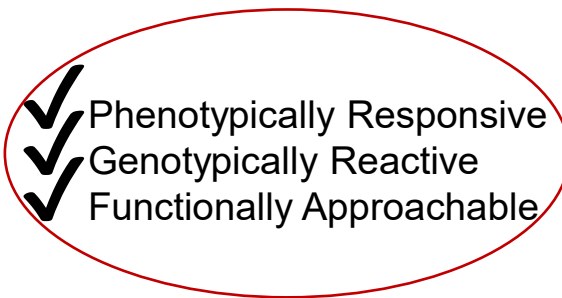A dedicated vertical offering Safety and Efficacy Testing as a Solution to the industry

Human Cell and Tissue In vitro Platforms Configured as Microphysiological Systems Concept

A Science Exchange registered portfolio
Supported drug discovery and preclinical research by supplying configured cell / tissue platforms - Validated use

Alternatives to existing systems
Complementing few existing systems
Configured in vitro platforms
Platform composition dictating the use case

Relevance
Meaningful modelling
Easy handling / predicting
No extrapolation of test read outs to human species

✓ Phenotypically Responsive
✓ Genotypically Reactive
✓ Functionally Approachable

TRANS-HSC   TRANS-MSC   TRANSCHYMAL™ - DP

TRANS -TT   TRANSKIN   TRANS-TR-B

Platforms with predictive capabilities

Peer reviewed concept developed as
Solution for testing Vaccine Human Neurovirulence Signals

NeuroSAFE

Offered as Solution has 2 components:

❖ <u>TRANS-MSC Unit</u> (A Transcell developed Transtoxbio residing Human Induced Pluripotent Stem Cell Configured In vitro Platform)
❖ Trained Software impregnated with AI/ML that can predict signals from test material treated TRANS-MSC

A non-animal, non-biopsy, cruelty-free, New Approach Methodology based Workstation solution for Vaccine and Pharmaceutical industry

FEATURES:

● An in-vitro HiPSC based configured human microphysiological platform

● A Digital trained platform

Enquire Now

www.transtoxbio.com

NeuroSAFE
Human Stem Cell Technology
&
Robotic Process Automation

Revolutionary product offering with in-built bandwidth to be integrated in the User's workflow – Robotic Process Automation
Reviewed by Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM)

Certificates & Patents

202241008032 TEMP/E1/9295/2022– CHE
Priority date: Feb 15th 2022 for IPO, USPTO and WIPO

A NON-ANIMAL CRUELTY FREE HUMAN RELEVANT WORKSTATION SYSTEM AND METHOD FOR TESTING NEUROVIRULENCE AND NEUROTOXICITY IN VACCINES
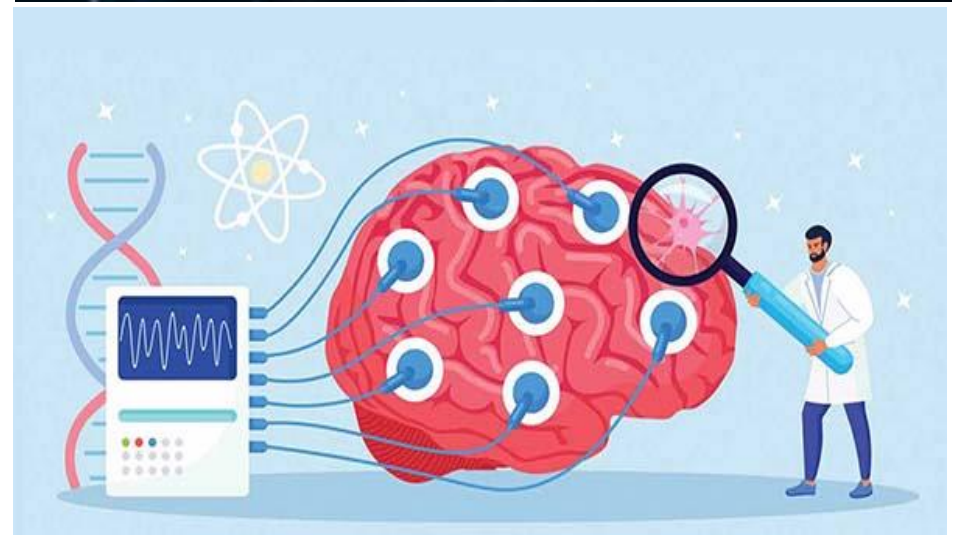
FIELD OF THE INVENTION

[0001]   The present invention generally relates to the field of any structural or functional mimic of neurotropic virus or viral component developed as vaccines. More specifically, the present invention relates to a system and method for cruelty-free testing of safety risks such as neurovirulence, neurotoxicity, and sterility in vaccines production process.
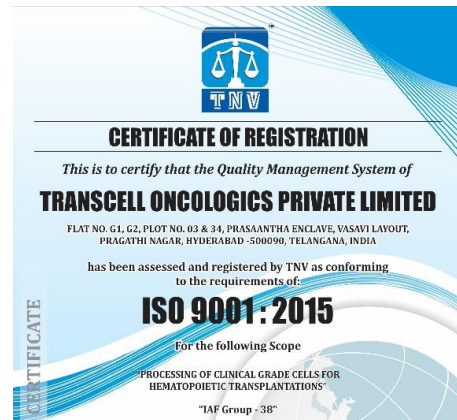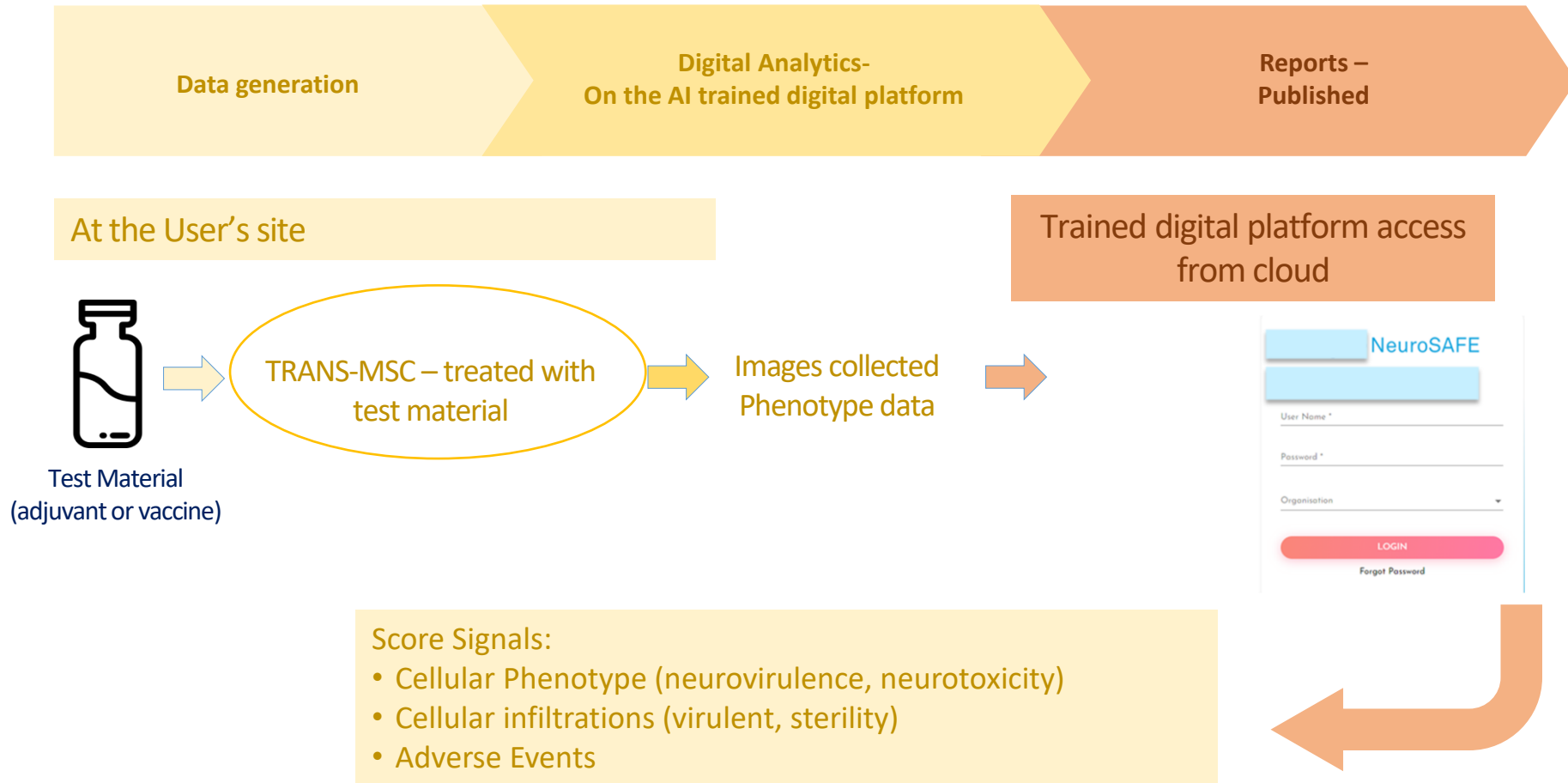
BENEFITS

- ✓ Compatible with all kinds of Covid-19 vaccines (structural or functional mimics of neurotrophic virus/viral particle)

- ✓ Suitable to other vaccines that are mandated to be tested for Neurovirulence

- ✓ Suitable in detecting microbial contaminations

- ✓ Can be adopted to detect Neurovirulence signals in Pharmacovigilance

NeuroSAFE –
Vaccine Testing – Scheme - Digitized

TRANSCELL Oncologics

| Data generation | Digital Analytics-
On the AI trained digital platform | Reports –
Published |

At the User's site

Trained digital platform access from cloud

Test Material
(adjuvant or vaccine)

TRANS-MSC – treated with test material

Images collected
Phenotype data

NeuroSAFE

User Name *

Password *

Organisation

LOGIN

Forgot Password

Score Signals:
- Cellular Phenotype (neurovirulence, neurotoxicity)
- Cellular infiltrations (virulent, sterility)
- Adverse Events

Leveraging Human Microphysiological System as a Model for Evaluating Safety & Efficacy of bio pharmaceuticals
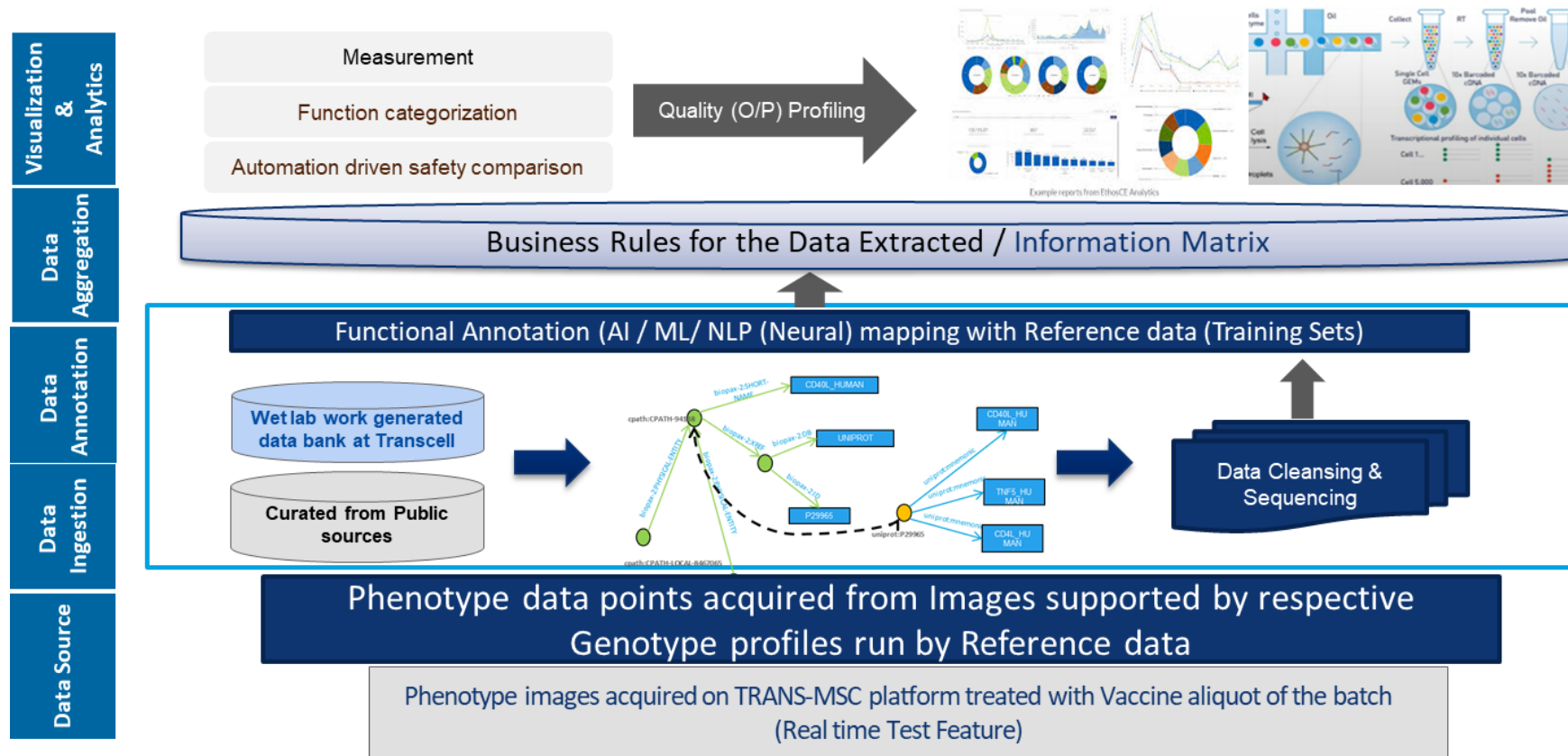
A comprehensive platform technology combining
cells / tissues configured human microphysiological platforms
with
Image and data analytics integrated in the workflow

*Next Gen Workstation Solutions For*

Safety & Potency Testing

Human cells in vitro system

AI and ML tools detecting safety signals
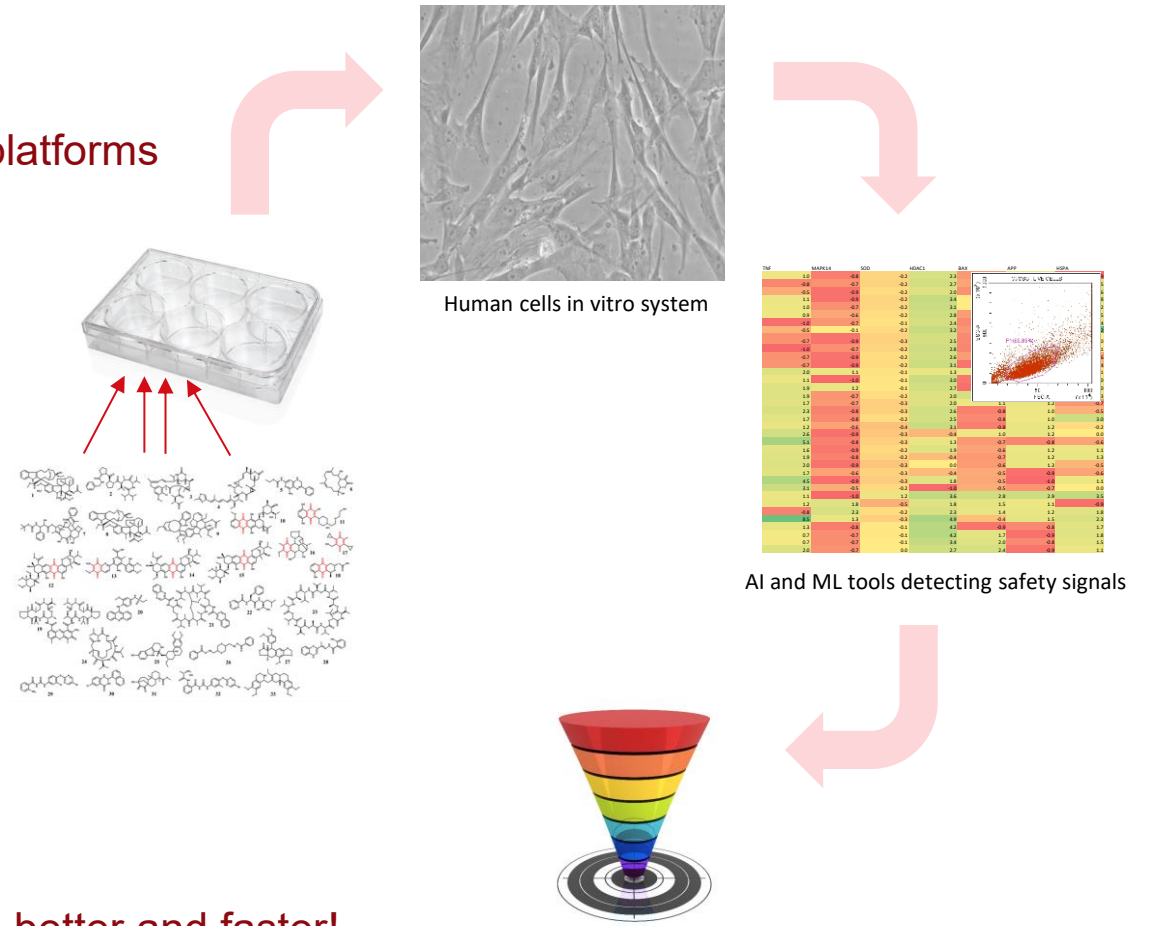
To understand your pharma and biopharma assets … better and faster!

Data Extraction, Mapping, Analysis, and Publishing

Visualization & Analytics
- Measurement
- Function categorization
- Automation driven safety comparison

Quality (O/P) Profiling

Example reports from EthosCE Analytics

Data Aggregation

Business Rules for the Data Extracted / Information Matrix

Functional Annotation (AI / ML/ NLP (Neural) mapping with Reference data (Training Sets)

Data Annotation

Wet lab work generated data bank at Transcell

Data Ingestion

Curated from Public sources

Data Cleansing & Sequencing

Data Source

Phenotype data points acquired from Images supported by respective Genotype profiles run by Reference data

Phenotype images acquired on TRANS-MSC platform treated with Vaccine aliquot of the batch (Real time Test Feature)

Built on TRANS-MSC (HiPSC based Invitro System)
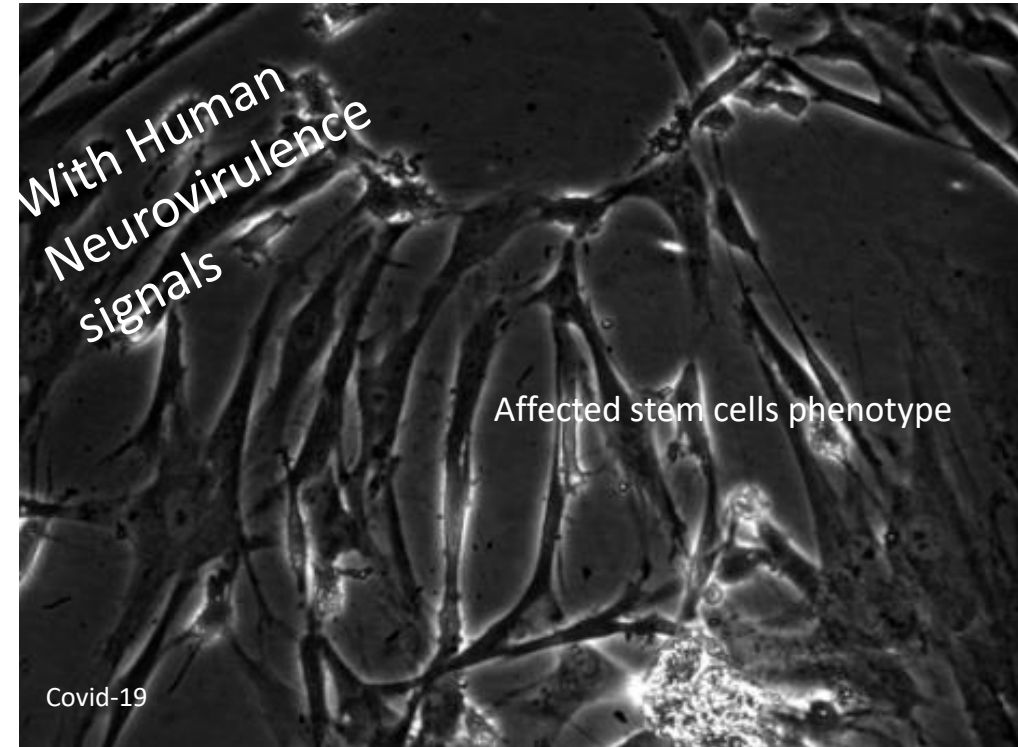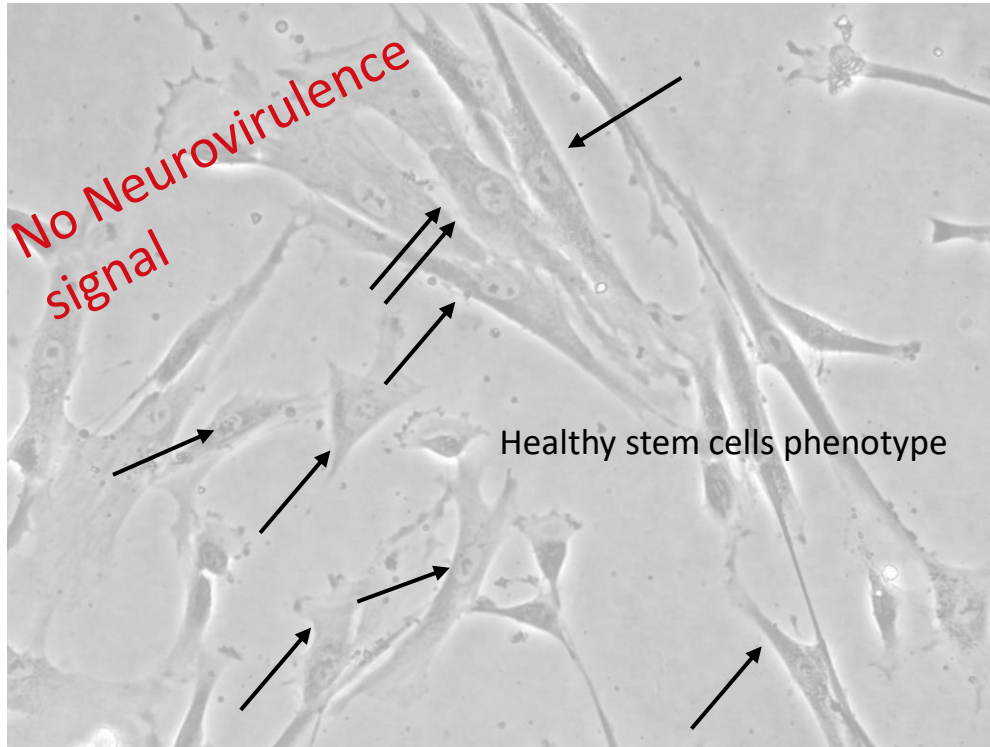## *A well characterized Human Cell Platform technology (Peer reviewed)*
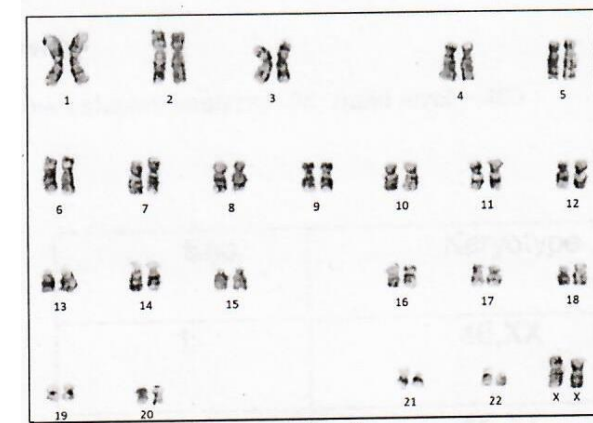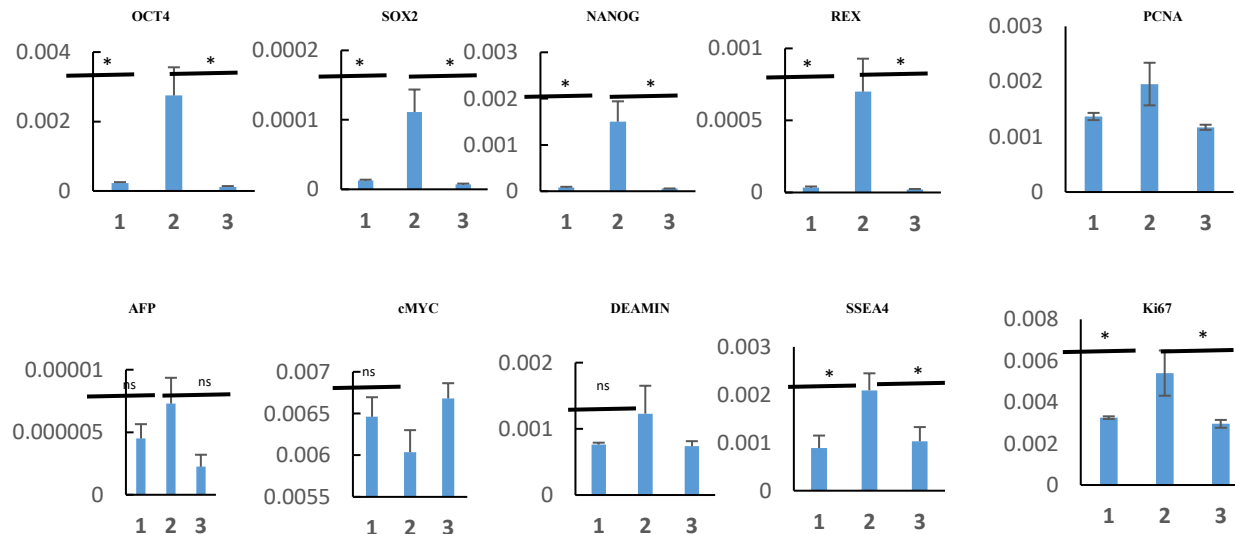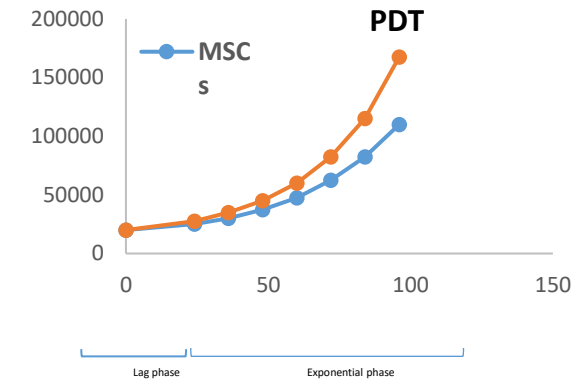
In vitro profiling of application ready human surrogate primary progenitor stromal cell fractions, Archives in clinical and biomedical research), June 2022

Transcell Oncologics work

NeuroSAFE – Visual Analysis of Cells after Exposure to Viral agents

No Neurovirulence signal

Healthy stem cells phenotype

With Human Neurovirulence signals

Affected stem cells phenotype

Covid-19

Training sets >3000 images captured and fed in to the AI prediction model as part of training

NeuroSAFE – Application Demo

❖Treating TRANS-MSC Unit with the test agent (Wet Lab [video](#))

❖Phenotype data acquired on treated TRANS-MSC fed into the trained Software to test predict signals of human neurovirulence – [Web app demo](#)

Training sets >3000 images captured and fed in to the AI prediction model as part of training

NeuroSAFE – Prediction Model – Generates Human Neurovirulence Signal Patterns

Follows WHO set Scoring method (Ref: SOP Version 2012; Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral). Proposed replacement of: TRS 904, Annex 1 and Addendum TRS 910, Annex 1. Expert Committee on Biological Standardization 2012. World Health Organization.

Grade 1: Cellular infiltration only (affected cells)
Grade 2: Cellular infiltration with minimal neuronal lesions (affected cells)
Grade 3: Cellular infiltration with extensive neuronal damage (affected cells)
Grade 4: Massive neuronal damage with or without cellular infiltration (affected cells)
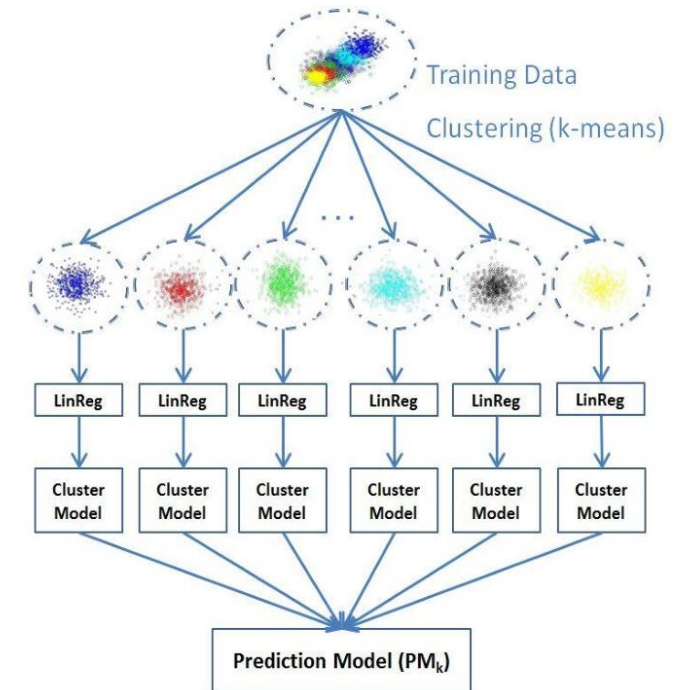
Training sets >3000 images captured and fed in to the AI prediction model as part of training

NeuroSAFE deliverables (human relevant data on TRANS-MSC) Cruelty free
For all vaccine candidates/vaccines developed on neurotrophic viruses

		Virus seed stock	Vaccine candidate (test agent 2)	Vendor specific Bovine Serum or any protein ingredient (test agent 3)	Adjuvant (test agent 4)
Method	Assay on TRANS-MSC of NeuroSAFE	Wild type and Vaccine candidate if it is attenuated or	DNA or mRNA or Protein		
Inverted Phase contrast microscopy	Phenotype changes				
Mitochondrial toxicity (Glu/Gal) assessment	Mitochondrial damage				
TUNEL kit	Apoptosis				
Wst kit	Cell death				
Immunoflurescence	Neurotropism				
Plaque assay	Viral replication		████████████████████████		
Whole Genome Sequencing and Bioinformatics tools like BLAST, Functional classification of neurotox genes altered	Genome Wide Screening				
Western blot	Human Receptor Mapping				
Passaging and gene expression	Viral Latency and Reactivation				
Stress modeled and gene expression	Safety Endurance				

NeuroSAFE deliverables (non-animal) – QC Metrics
Cruelty free
For all vaccines developed on neurotrophic virues

NeuroSAFE Acclimatization Period (NAP) @ the User's site 1. Generate atleast 5000 morphologies on TRANS-MSC treated with the test agent at 5 different concentrations (very low, low, low medium, high medium, high) and generate all possible human neurovirulent signals. 2. Train the NeuroSAFE software machine with the test agent specific human neurovirulent signals to customize the application as QC check point in the vaccine production process	2-3 months
Onboarding, Integration, User acceptance in the production workflow	
Real time implementation	contract period
Annual maintenance	contract period

NeuroSAFE deliverables (non-animal) – QC Metrics
Cruelty free

Test Name	Test for Extraneous Agent
Detection of Bovine, porcine Viruses	Haemagglutination Agents, Avian leucosis viruses other extraneous Viruses
Tests for freedom from Detectable SV40 sequences	Simian Virus
Detection of Human viruses	Human Retrovirus family (5 types)
Mycobacteria by culture method	Mycobacterium spp.
Mycoplasma detection by culture method	Mycoplasma pneumonioae

Thank You

NeuroSAFE

Supplementary Slides

NeuroSAFE   Filling the gaps

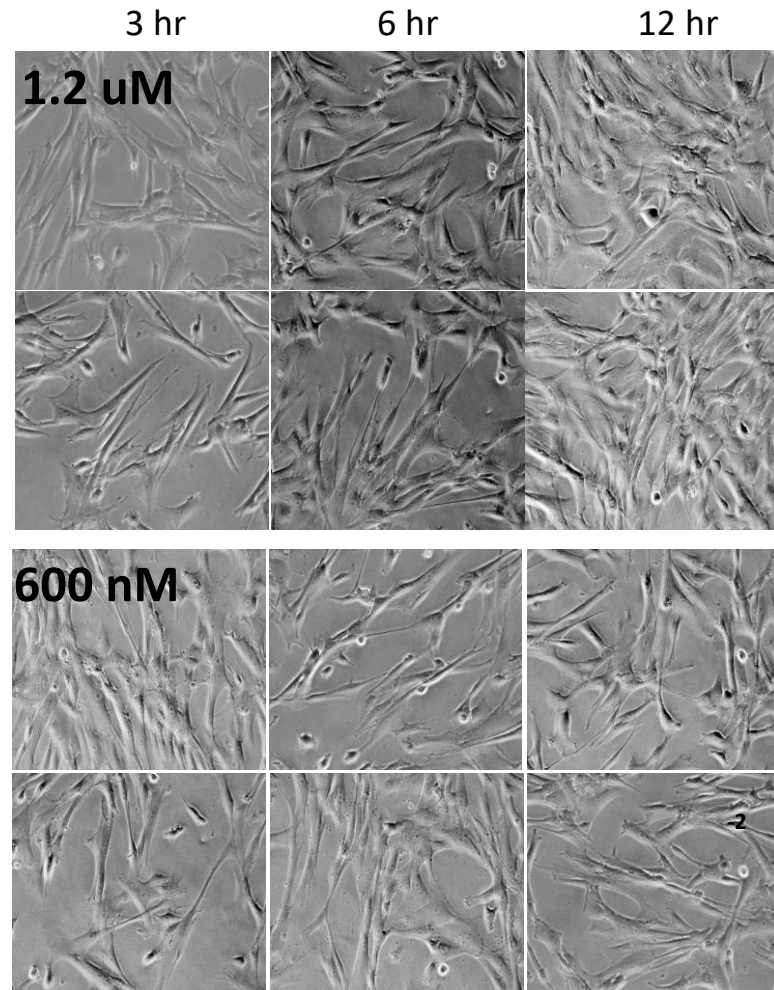|	Addressed while developing NeuroSAFE
Source of the cell based platform	Source is abundant with established protocols to obtain large scale primary, progenitor population
Nature of the cell based platform	Characterized HiPSC
Test endpoints	Cell infiltration, Phenotypes barcoded with corresponding neural genotypes representing functional omics to pick human Neurovirulence signals
Heterogeneity of the cell based platform	Quiescent Phenotypes achieved in the training data sets while AI and ML create corresponding heterogeneous patterns
Heterogeneity in methods for differentiating cells of the Central Nervous System	TRANS-MSC is supplied as a component in the Solution being offered
Suitable for Vaccines developed for human Neurotrophic viruses, novel adjuvants	Suitable for structural and functional mimics of viral subunits with phenotype perturbations taken as fundamental data set

TRANS-MSC – In Response To Toxins

Genetically characterised cell death parameters

3 hr   6 hr   12 hr

1.2 uM

600 nM

TRANS-MSC Phenotype – Toxin Exposed

Protein level

TMPRSS2

Lane details:
1. MSC
2. TRANS-MSC
3. TRANS-MSC-N
4. Vero
5. Vero

ACE2

β actin
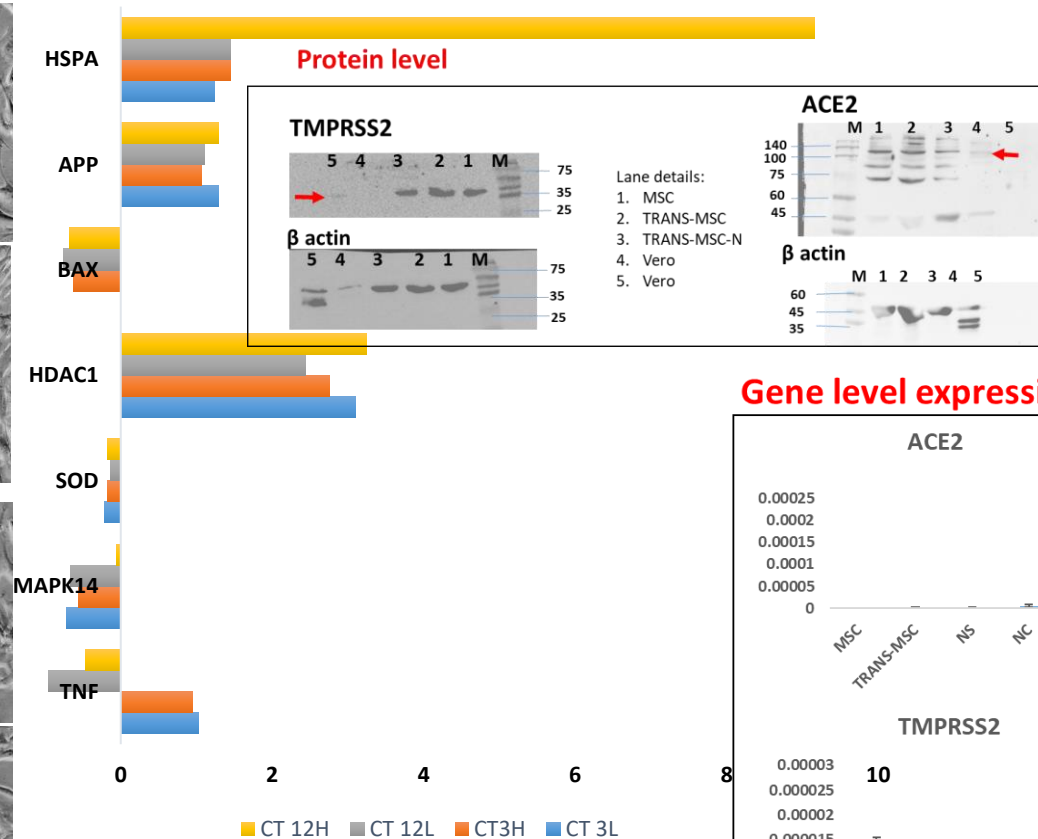
Gene level expression

ACE2

TMPRSS2
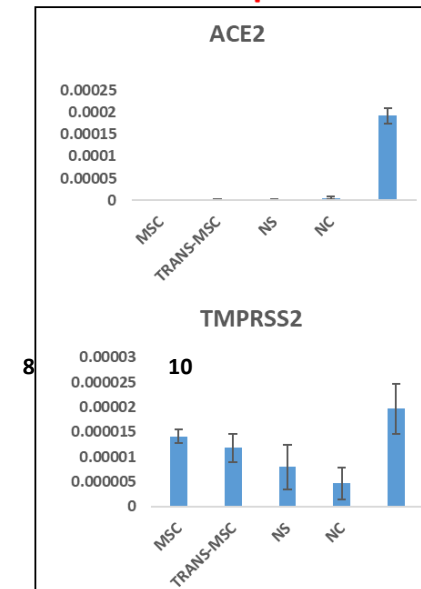
ACE2 has documented role in SARS-CoV2 and HIV infections

Legend: CT 12H, CT 12L, CT3H, CT 3L

Validating

Wet lab work generated Benchmark data sets

Tetanus toxin
An example of Apoptosis
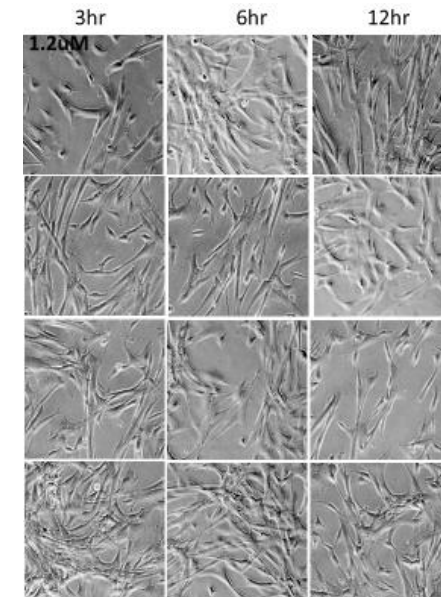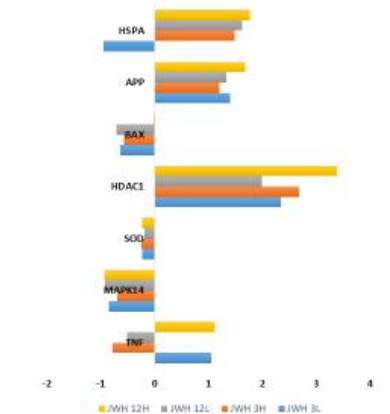
>1000 pictographs (phase contrast
micrographs of virus, vaccine,
bacteria, toxin treated
TRANS-MSC)

>250 neurotoxic gene sets
that have established role in
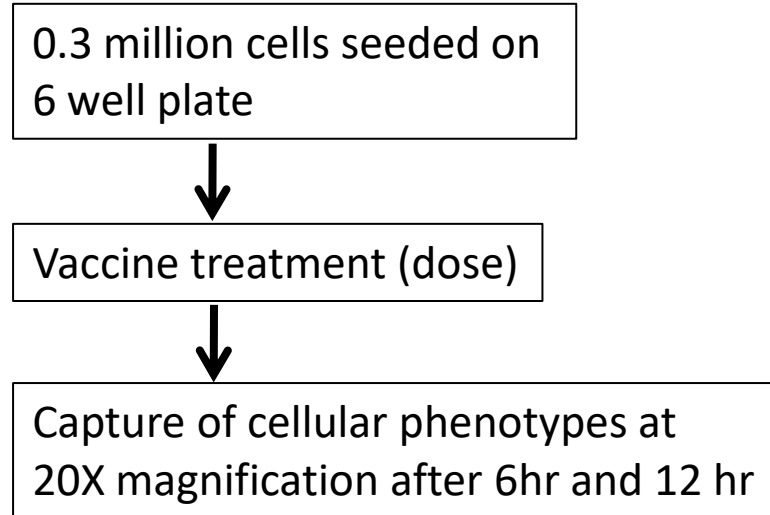chemical, bacterial, viral
Induced neurotoxic
pathways

5 different concentrations and
3 different time intervals

JWH018
An example of Necrosis

Ensembl , nextprot, pubmed
Curated data

Testing of vaccines on NeuroSAFE

0.3 million cells seeded on 6 well plate

↓

Vaccine treatment (dose)

↓

Capture of cellular phenotypes at 20X magnification after 6hr and 12 hr

The scoring system followed is based on WHO MNVT system:
- Cell infiltrations signal
- Affected cells signal

Polio IP   YFV

Covaxin   Covishield