



# Drug safety and discovery with Human iPSC-Neuron and Artificial Intelligence

**TechnoPro R&D**

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Research services: Biotechnology and chemistry  
Research centers: 3 sites in Tokyo areas and Kobe  
Established: October, 1988

Leadership

- Yasuji Nishio: President and Representative Director, TechnoPro Inc.
  - Masami Hayafune: Representative director, TechnoPro R&D Company (TRD), TechnoPro Inc.
  - Directors of Product Planning, Lab operation and HR at TRD
- Scientific Advisory Board: Professor Ikuro Suzuki, Ph.D.

**Gene/Cell**

Expression vector  
transduced cell  
line, gene analysis

**Protein**

Expression of  
recombinant protein  
Protein Identification  
Enzyme assay

**Drug screening**

System validation  
Biochemical assay  
Cell-based assay

**Cell culture**

Toxicology  
Skin-Lightening,  
Moisturizing, Sebum

**Material**

compound,  
polymer synthesis

**Peptide**

synthesis,  
bio-activity assay

**Analytical  
chemistry**

**Animal  
models**



■ **MEA research service of a microphysiological system**  
with public private partnership

- **Neuroscience and Drug Discovery** (MEA: Micro Electrode Array)
- **Research achievement: 3 big pharmaceuticals, 1 Biotech, 1 Academia in one year and half**

# ■ The Research Technologies: COUs

## (1) Human cells

- Side effect and efficacy **prior to** clinical study
- Critical evidences for **stage-up** of research projects
- Specially selected iPSC-neuron screened from **>50 neuron lines**
- Variety of human iPSC-neuron lines of cortical, motor, sensory
  - ➔ Neurodegenerative diseases models

## (2) Drug screening and evaluation of safety/efficacy

- Strong **time-resolution** and fidelity compared with optical imaging, Ca<sup>++</sup>
- Real-time/long term neuron culture in **non-invasive/non-toxic**
- Phenotypic screening in short-term ➔ **prioritization** of lead compounds
- Recording neural activity with appropriate MEA devices to researches
  - ➔ Hi-throughput, CMOS-MEA, Low-frequency wave

## (3) Rich pathways to neural function study

- Extract parameters from fundamental data of raster plot, burst firing, frequency
- Unique/independent/patented evaluation with AI and algorithm of Multivariate analysis with enormous parameters
- functions for ion channels and receptors (Axon propagation, electric imaging )
- High-content imaging: iPSC-neuron differentiation and drug safety/efficacy

## (4) Difference, relativity and extrapolation to vivo/clinical

- Quantitative toxicity evaluation ➔ Replacement, reduction and refinement  
**Alternatives to animal testing** w/ MEA, minimizing research period, down-sizing synthesized samples

## ■ Contexts of use : Human neurons

### ■ iPSC differentiation

- Induction of differentiation to neurons with gene edited cells or from healthy donor/patient cells ➡ Disease models
- Electric action potential: Recording with Micro-electrode array, multivariate analysis and artificial intelligence
- Optical measurement with hi-content image analyzer
  - Combined study with MEA
  - Tracking process of iPSC differentiation and function
    - Morphology of axon elongation and synapse conformation
    - Expression of neural markers, ion channels and receptors



### ■ Drug safety/efficacy with disease models

Gene-edited iPSC-neurons and iPSC-neurons from healthy donor/patient

- (1) Alzheimer's disease neuron (Presenilin1-mutation)
- (2) ALS-related neuron (C9orf72 repeat expansions, SOD1-/- KO)
- (3) Spinal cord neuron
- (4) Sensory neuron (Dorsal root ganglion) for neuro-pain assay through TRP channels, neuronal propagation and drug efficacy hypersensitivity administering cancer drug

# ■ Contexts of use : Drug screening and evaluation

## ■ Drug phenotypic screening

- With positive chemicals affecting ion channels and receptors
- Evaluate massive samples in short-term with high throughput  
➡ for the second screening



nature

SCIENTIFIC  
REPORTS

nature

SCIENTIFIC  
REPORTS



HESI



## ■ Drug safety and efficacy Optimization and Prioritization

■ **Fundamentals** : Physiological maturation and drug responses of human induced pluripotent stem cell-derived cortical neuronal networks in long-term culture (Scientific Reports 2016 May 6:26181)

### ■ Seizure-inducing risk

- Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an MEA system (Scientific Reports 2018 Jul 8:10416)
- Can we panelize seizure? (Toxicological Sciences, 179 (1), 2021, 3–13)

### ■ Neurotransmitters and LTP/LTD

- Induction of long-term potentiation and depression phenomena in human induced pluripotent stem cell-derived cortical neurons (BBRC Jan. 2016 469:856)
- Impact of Sleep–Wake–Associated Neuromodulators and Repetitive Low-Frequency Stimulation on Human iPSC-Derived Neurons (Frontier Neurosci. May 2019 13:554)

### ■ Axon propagation on neuron network Electrical Imaging

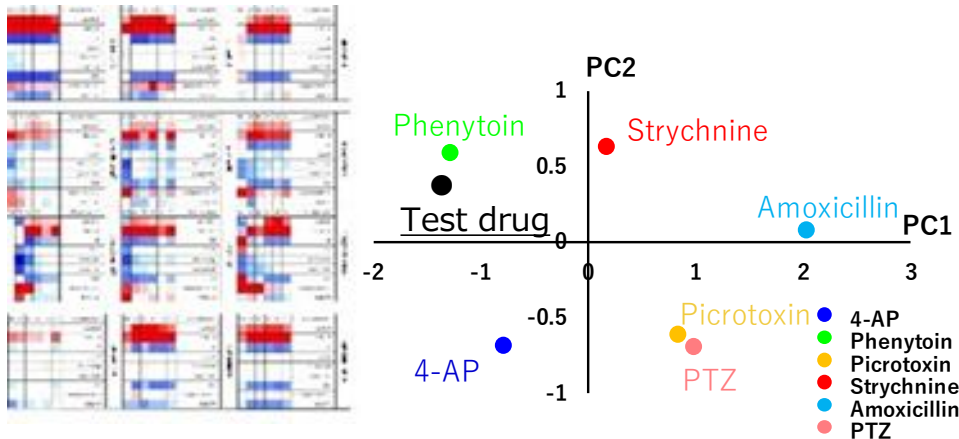
- Versatile live-cell activity analysis platform for characterization of neuronal dynamics at single-cell and network level (Nature Communications 2020 11:4854)

## ■ Contexts of use : Diverse analyses for neural function

## ● PCA: Detecting function/toxicity/efficacy

- Inductive Logic, Statistical regression analysis
- Relativity/extrapolation to vivo/clinic precisely

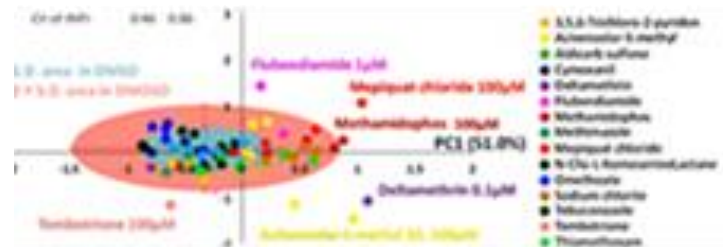
## ■ Difference/similarity to positive controls



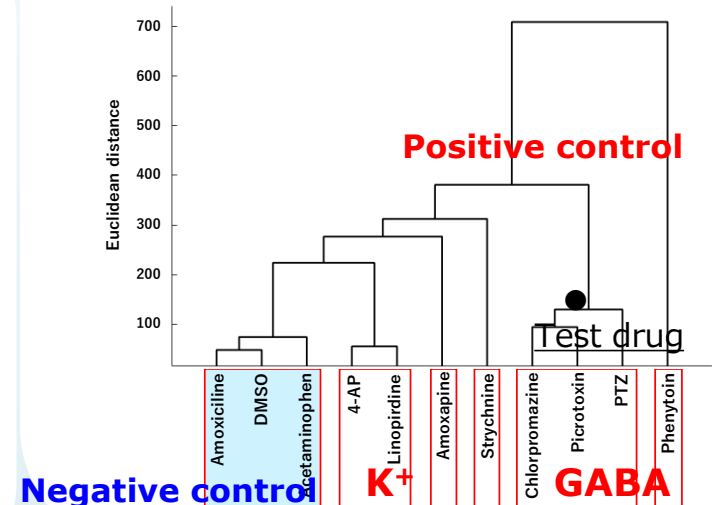
Total synaptic action potential → Distinguish synaptic input of specified ion channels and receptors → Reveal functional mechanism

## Put parameter sets of **negative controls**

- low risk: one SD in blue
- Mid-risk: Two SD in red
- Hi-risk: Out of ovals



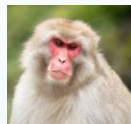
- **Clustering**: Functional classification for screening and prioritization





# Contexts of use : Difference, relativity and extrapolation

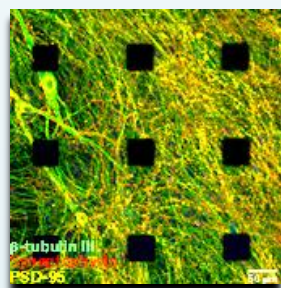
AI-drug discovery/  
Translational Research



- **Difference** : Detect unexpected toxicity and estimate
  - MEA detects toxicity of drugs measured non-toxicity in vivo rodent but being toxic in vivo primate.
  - ➔ Estimate an appropriate clinical dose

- **Relativity**: In safety study, toxic concentration in CSF in vivo is identical to that in mouse neuron culture of MEA
  - ➔ Quantitative toxicity evaluation, animal study reduction, minimizing research period and synthesized drugs

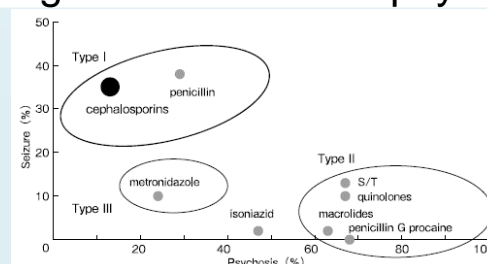
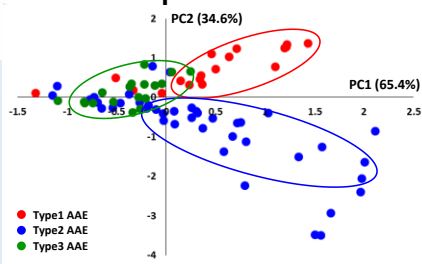
- MEA: seizure-like phenotype in human iPSC-neuron and rodent primary cultures
- In vivo: Primate and Rodent at icv single-dose study (seizure, tremor, vital sign)



Human/rodent  
neuron network  
in vitro



- **Extrapolation to clinical** : Reconstitute drug classification for adverse events of AAE with MEA of human iPSC-neuron
  - MEA predicts clinical risks of drugs for seizure and psychiatry



AAE (Antibiotic Associated Encephalopathy) is a drug-associated disorder dosing antibiotics. The symptoms classified of three types by severity of seizure and psychiatry.

# ■ Cell Culture

# Process

Culture

Record

Analysis

- **Neural network in co-culture**

- Excitatory cortical neuron/inhibitory interneuron/astrocyte
- The network matured in a month for total 2-3 mo for the evaluation

1. **50+ Human iPSC-Neurons (and cardiac cells)**

Glut/GABA/Chol/Dopa/DRG/Motor neurons in evaluation cases with 1500 compounds

2. **Rodent primary neurons and brain slices**

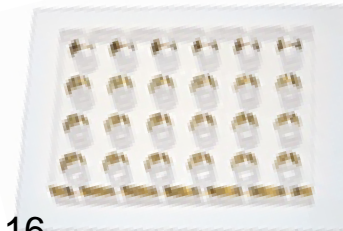
(Cortex, cerebellum, brain stem, DRG)

3. **User's cells and lines of a biobank**

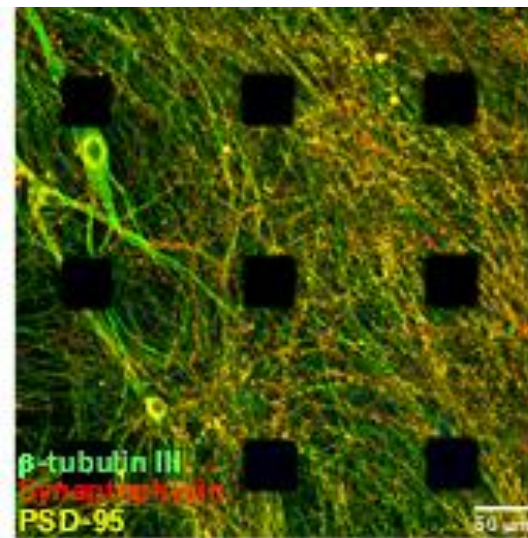
Available to induce human neurons from iPSC



24-well/384 electrodes  
(16 electrodes/well)



Modality: Small Molecule,  
Oligonucleotide, Antibody,  
Protein, Cell, Tissue



G Tubulin beta III/Tuj1, Neuron  
R Synaptophysin, Presynaptic vesicles  
Y PSD-95, Synaptic region



# ■ Recording Responses

Culture

Record

Analysis

**Measure action potential** of the neural networks  
with the MEA system

- Samples applied at 5 doses and 6 replications with +ve controls

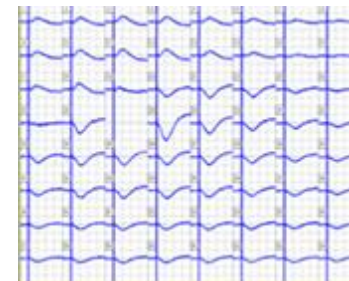
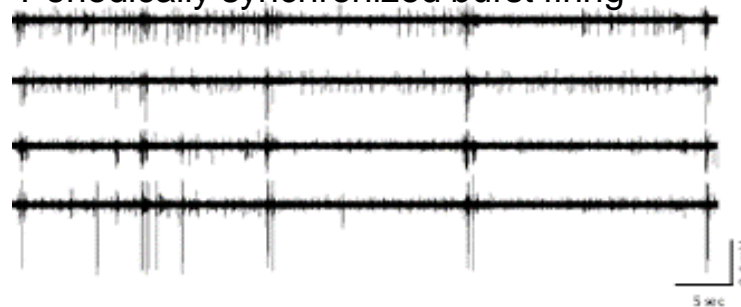
The first report of MEA analysis with human iPSC-neuron in 2014



**Lead evaluation of drug safety and efficacy internationally**  
with precisely detection of **drug response at lower by MEA**  
than by cell death assay in safety

**Recording** Action potential of Na<sup>+</sup> influx on an extracellular field

Periodically synchronized burst firing



# ■ Analysis 1: Action potential Visualization

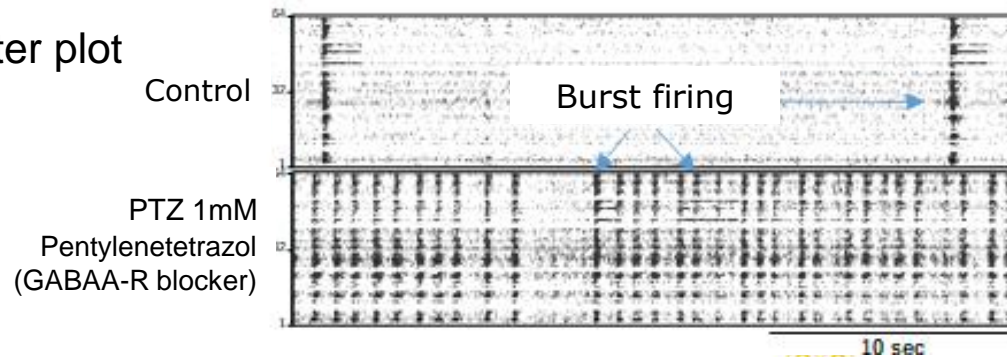


## (1) Histogram Analysis

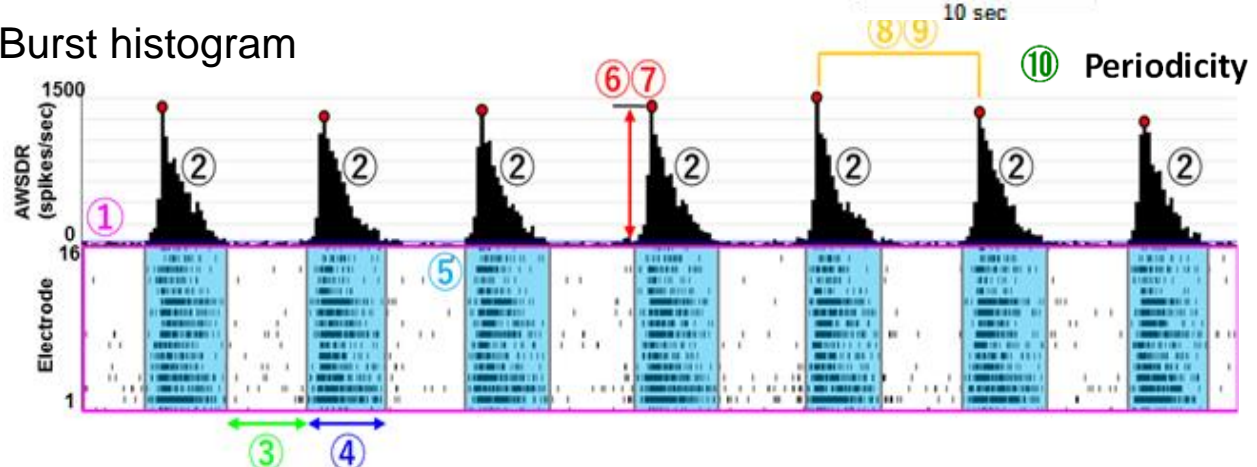
- ➔ Extract targeted parameters for benchmark of analyses as being difficult to reveal drug functions with a single parameter

### ■ Drug response in neural network cultured on MEA

#### • Raster plot



#### • Burst histogram



1.Total spikes, 2.Nr.of SBF, 3. Inter Burst interval, 4. SBF Duration, 5.Spikes in SBF, 6.Max Frequency (MF; Spike peak), 7. CV coefficient of variation of MF, 8.Inter MF Interval (IMFI), 9.CV of IMFI, 10. Periodicity

# ■ Analysis 2: Multiple classification

Culture

Record

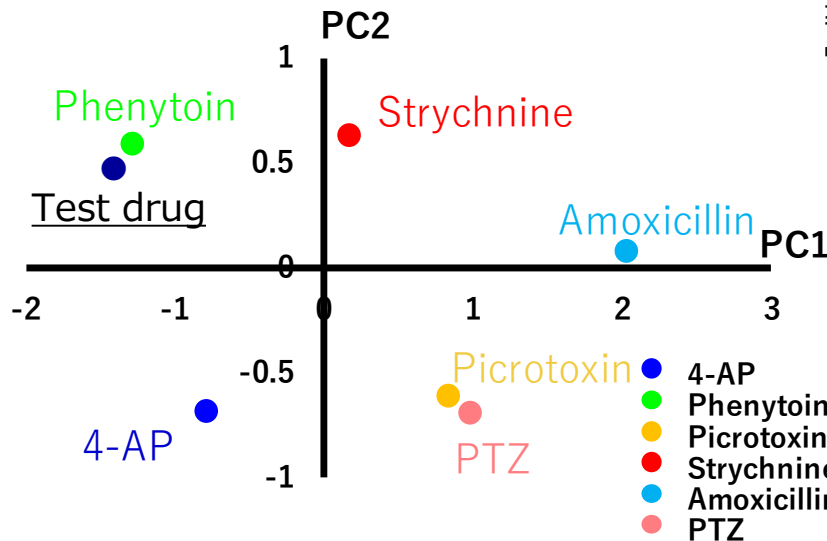
Analysis  
1&2

## (2) Multivariate Analysis (Patented)

- PCA (Principal component analysis) / Cluster analysis with massive parameters > 90
- Cumulative data, **200+** physiological substances

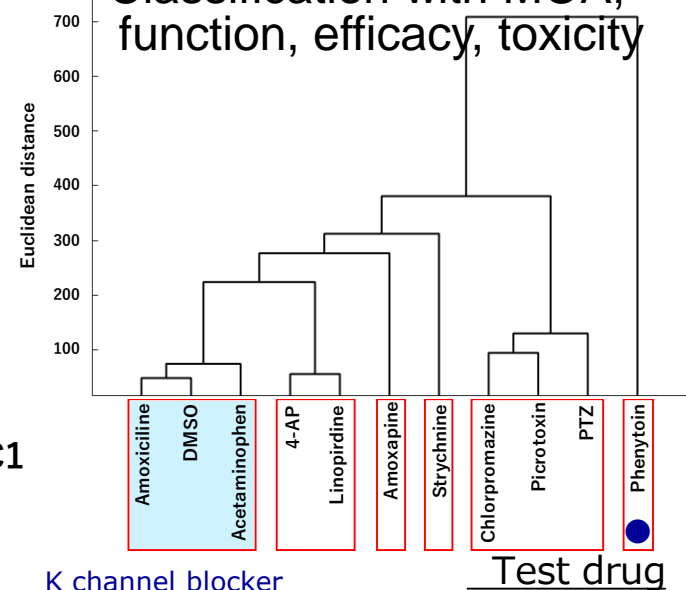
Adding to Analysis 1...

**PCA:** Plotting detection of similarity to +ve controls



## Cluster analysis:

Classification with MOA, function, efficacy, toxicity





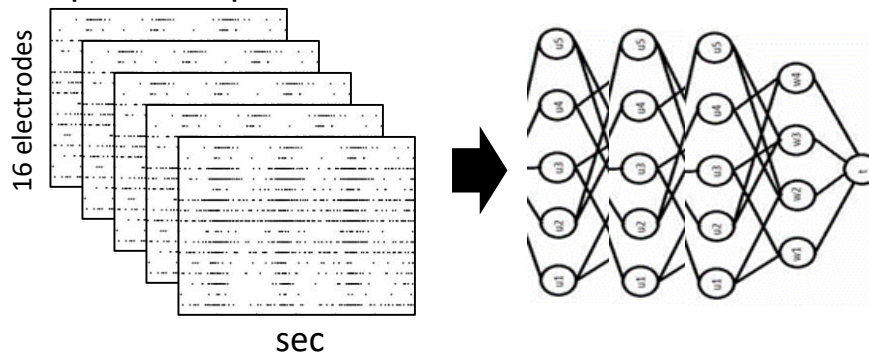
# ■ Analysis 3: Artificial Intelligence

## (3) AI analysis

- Distinguish MOA, function, toxicity **undetected** by the multivariate analysis (Patented)

Adding to Analysis 1 and 2...

Spike stamp table



sec  
[Deep Learning] → Positive control drug

- Pattern learning of spike stamp tables w/ 4096 ch.
- Weighted recognition, and Extract feature quantity
- Pile up multi-layers of algorithm for recognition model equation
- Generate recognition algorithm

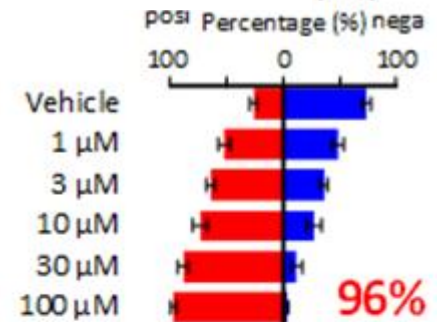
↓  
[Identification] → Test drug

- Identify similarity and difference of
- Classify function, efficacy and toxicity risk

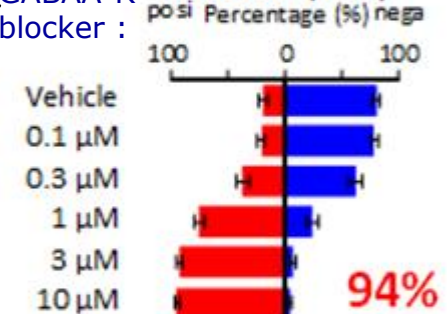
e.g. Recognized convulsants in dose-dependent

Neurotransmitter  
Release Enhancer

**Linopirdine (n=6)**

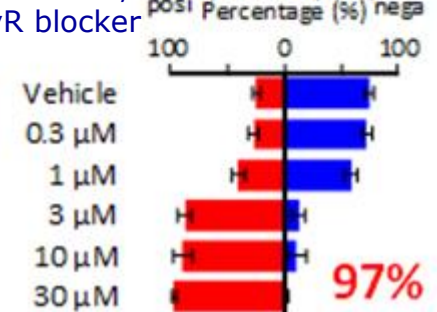


**Picrotoxin (n=10)**



Convulsant,  
GlyR blocker

**Strychnine (n=9)**



Culture

Record

Analysis  
1+2+3



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