"Applications and Project Needs" includes having useful DATA and TOOLS: fitting high throughput/high content screening of engineered nanomaterials to a safe innovation approach

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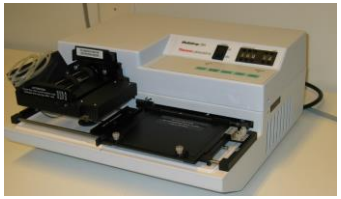
Needs of platforms for model standardization and high-throughput screening (HTS) of engineered nanomaterials toxicity

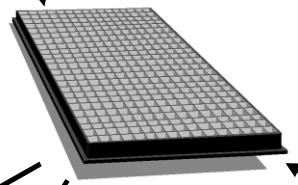
Hamilton STAR
-8+96 channel liquid handling

MultiDrop 384 -plate filler

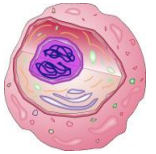


1) Pipet 5 µl of nanomaterial dilutions

2) Add 45 µl of suspended cells



384 well plates



3) Incubate (and image) 72 hrs

4) Add cell phenotype stains & incubate

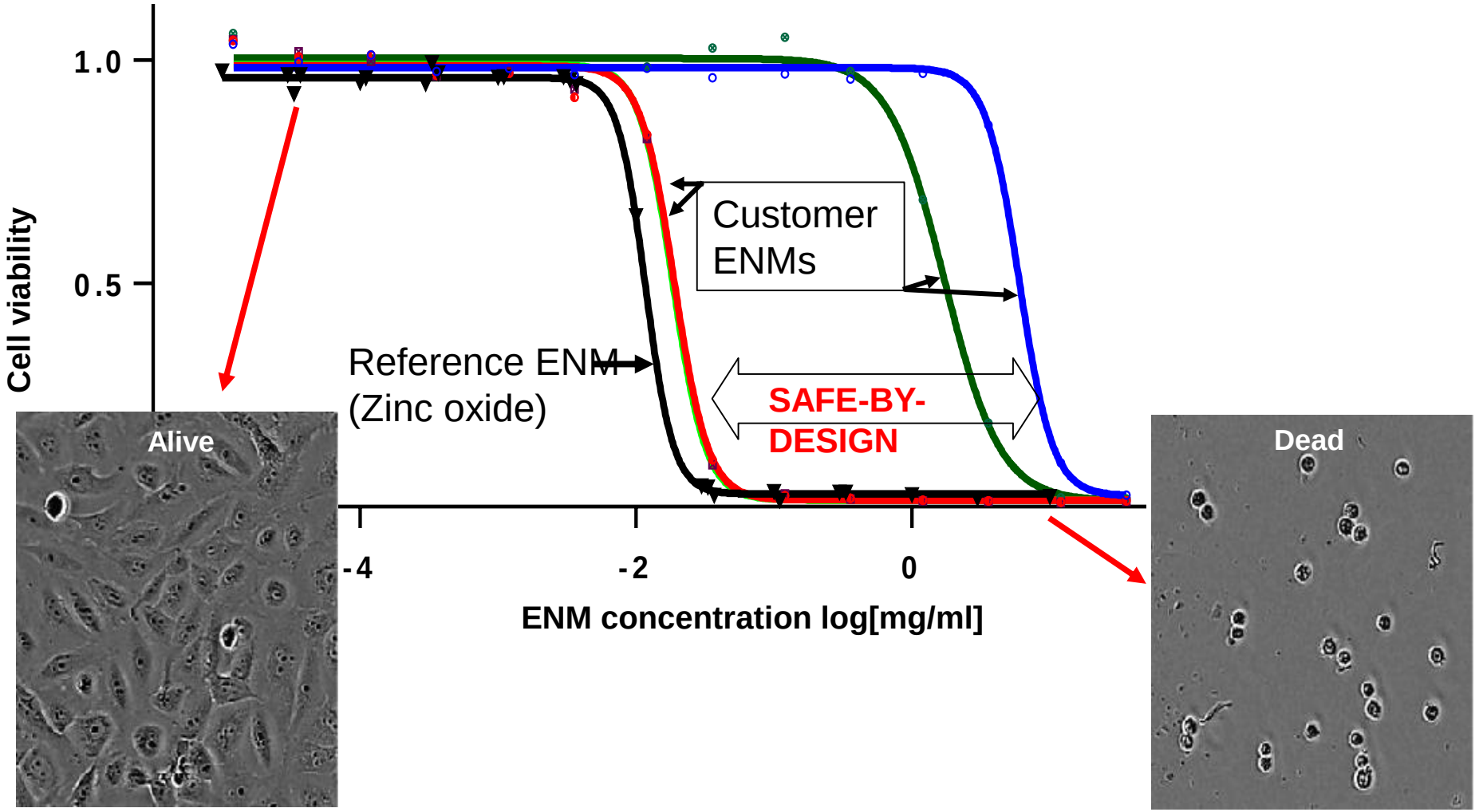
6) Luminescent measurement of ATP content /CellTiter-Glo assay

Perkin Elmer Envision multilabel plate reader

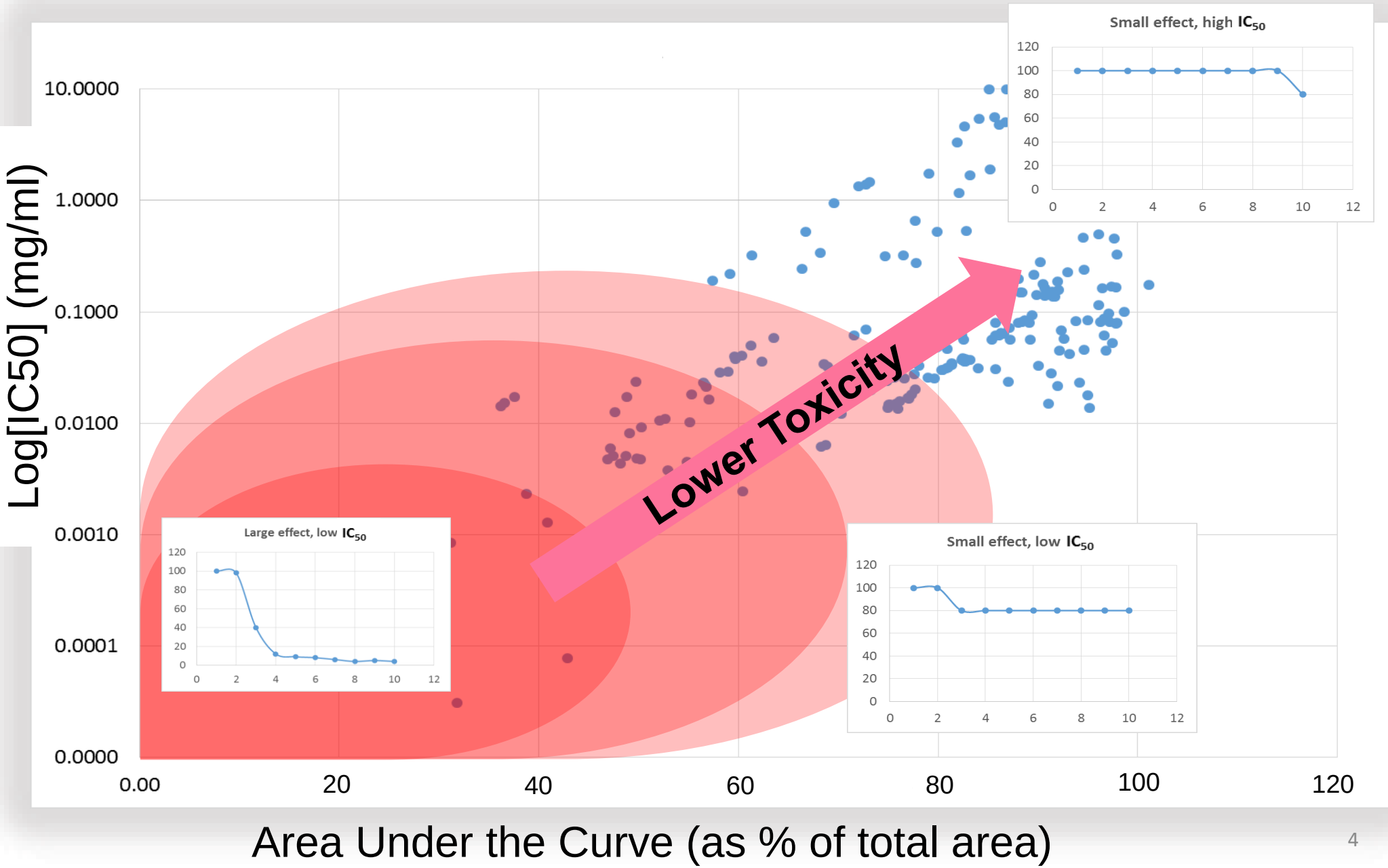
5) Timelapse-based assessment of cell shape and plate confluency level

Essen BioScience IncuCyte for imaging endpoint

Need of ranking of customer ENMs versus a common commercial reference ENM under a “safe-by-design” principle



Need of safety-by-Design Ranking: Area Under The Curve vs. IC50



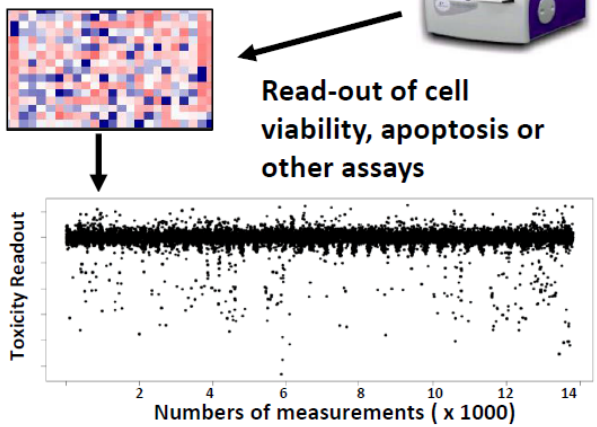
Need of tiered approaches: from HT screening of many agents to genomic profiling analysis of the selected few

Cell-based HT functional screen

Multiple chemicals & nanomaterials

Multiple concentrations

Robotics-based screening (384 wells)



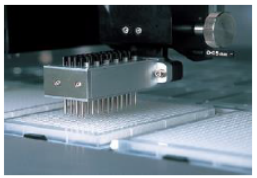
Read-out of cell viability, apoptosis or other assays

Cell-immunostaining microarrays

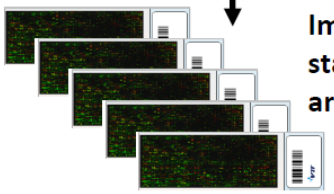
Multiple chemicals & nanomaterials

Selected concentrations

Lysing of cells from assays



Spotting of lysates to nitrocellulose filters

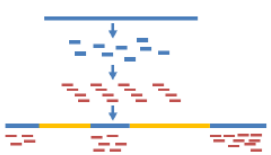


Immunochemical staining of the arrays

Gene expression profiling and bioinformatics processing

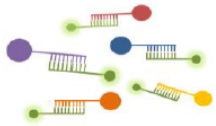
Selected chemicals & nanomaterials

Selected concentrations



RNA-sequencing

or



Reduced feature set assays

Quality control and normalization

- Dose-response analysis of gene expression alterations
- Similar gene patterns (clustering)
- Over-represented gene ontology categories and pathways
- Networks of functionally related genes
- Visualization and interpretation

Cell-based screening Readout

Basic cellular toxicity endpoints (2-4) related to:

- Cell viability, proliferation, cytotoxicity (e.g., CellTiter-Glo®, CellTiter-Blue®)
- Apoptosis (e.g., Caspase-Glo® 3/7, 8, 9)
- Membrane integrity tests or cell number (e.g., CellTox™ Green)
- Reactive oxygen species (e.g., ROS-Glo™ H_2O_2 assay)

Lysate Microarray Readout

Multiple (30-60) endpoints for generation of broad toxicity profiles related to:

- Apoptosis (cleaved PARP), cell cycle (e.g., Ki67), DNA-damage or other stress responses (e.g., H2AX, JNK2), aberrant differentiation, (e.g., vimentin) and inflammation (e.g., NF-κB)
- Other specific toxicity pathway markers (e.g., Nrf2/ARE, MTF1)

Bioinformatics Readout

Gene ontologies, molecular networks and single genes (>100) related to toxic mode-of-action

- Use of genomic signatures (biomarkers) to study adverse effects of chemicals
- Modeling of the toxic response
- Connectivity mapping (to find similar profiles) in a database of well-characterized compounds
- Integrative analysis across all assay types

Adapted from: Kohonen et al. Basic Clin Pharmacol Toxicol. 115:50-8 2014; Grafström et al, Altern Lab Anim. 43:325-32 2015

Need of new concepts for applying Omics in Safety Evaluations

Modelling together **large collections of gene expression and high-throughput cellular screening profiles** (i.e., «Big Data») should generate a first attempt of **toxome** description

Such a description should be able to serve as a **«Predictive Toxicogenomics Space (PTGS)»** as it should capture **toxicity mechanisms and pathological effects**

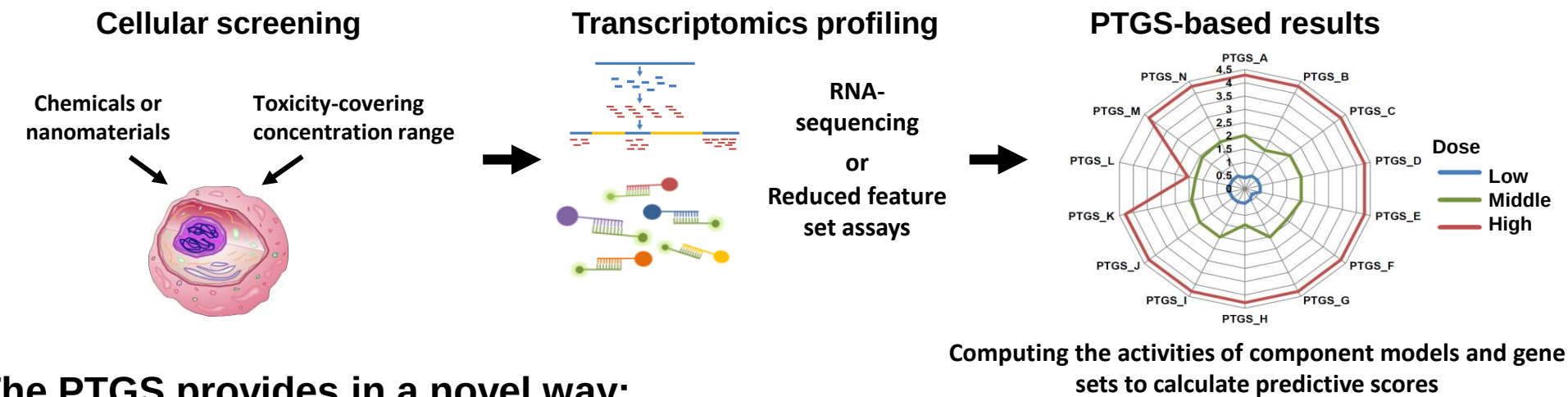
Bioinformatics-based validation against existing and coming data sets should prove the extent of usefulness of a PTGS for:

predicting Key Events for cellular and organ (e.g., liver) toxicity effects,

analyzing dose-dependent relationships

all to be useful to **Adverse Outcome Pathway (AOP)** studies**.**

Summary of the PTGS safety scoring concept



The PTGS provides in a novel way:

- A virtual cellular toxicity/non-toxicity probability estimate intrinsic to the omics-data
- New genes, mechanisms and concepts to the toxicogenomics field, accounting for existing cellular toxicity reactions
- Mechanistically validated calculation of NOEL/LOEL/toxic exposure thresholds for agent effects
- Grouping of chemicals into mechanistically similar classes for read-across safety assessment,
- Coverage of adverse outcome pathways-coupled toxicity effects involving multiple transcription factors/co-regulators, e.g. tumor suppressor 53,
- Probabilistic prediction of liver toxicity and pathology, including severity grade, from data obtained in cultured cells (e.g., rat/human hepatocytes) and laboratory animals (e.g., in rats)
- Prediction of dose/concentration in blood causing human drug-induced liver injury (DILI) from hepatocyte experiments is superior to, and complementary to, existing tests on the market.

Conclusions-Adhering to Future Data and Tool Needs

- **ENMs safety evaluation is achievable at 384/1536-well formats using the human lung epithelial cell line BEAS-2B**
- **Cell density, exposure time, culture with or without serum, dispersion protocols, storage stability, dilution effects, etc. can be rapidly assessed and integrated into standardized HT testing protocols**
- **ENMs demonstrate dose-dependent toxicity over a broad range of concentrations; the HT analyses consider possible assay interferences**
- **HTS-generated results agree with published results under lower throughput**
- **Time lapse imaging serve to validate the viability/toxicity assays**
- **Combined HTS, Array, and Omics-based approaches form tiered approaches to ENM safety evaluation and "bioidentity" definition**
- **Overall, HT/HC technologies are key to rapid knowledge generation, being a systems biology-based safe innovation approach to functionalization of ENMs**

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