Electronic Supplementary Material (ESI) for ChemComm.


*Supplementary information*

# Cytotoxicity of arsenic trioxide in single leukemia cells by time-resolved ICP-MS together with lanithanide-tags

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## Experimental Section

### 1. Materials and Reagents.

HL60 and NB4 cells were kindly provided by Dr Wai-Choi Tse (Department of Medicine, the University of Hong Kong).

The following chemicals and reagents are purchased from Sigma-Aldrich (St. Louis, MO, USA): Arsenic(III) oxide (purity, ≥99.0%); $Nd_2O_3$, $Dy_2O_3$ (purity, 99.9~ 99.99%); Retinoic acid (purity, ≥98%); cisplatin (purity, 99.9~99.99%); Annexin V-FITC apoptosis detection kit; Monoclonal Anti-CD11b−FITC.

Mouse Anti-Human CD11b (ProSpec); Annexin V from human placenta (Tin Hang Technology Limited); RPMI-1640 medium, fetal bovine serum (FBS), penicillin Streptomycin Sol were purchased from Life Technologies; Amicon Ultra-0.5 Centrifugal Filter with a molecular weight cut off at 3 kDa (Millipore). The deionized water (18.2 MΩ·cm) was used throughout the experiments.

### 2. Apparatus

A quadrupole-based inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 7700, Agilent Technologies, CA, USA), equipped with a glass concentric nebulizer and an impact spray chamber; and confocal Laser Scanning Microscope, Carl Zeiss LSM 710 NLO were used in this work.

### 3. Experimental procedures

### 3.1 Cell culture and treatment

Two different leukemia cell lines, NB4 and HL60 were used in this work. Cells were cultured in RPMI1640 medium supplemented with 10% FBS and 1% penicillin–streptomycin solution (37 ℃, 5% $CO_2$). For single cell analysis, cells were harvested by 3 repetitions of centrifugation (1000×g and 5 min) with 0.01M ice-cold phosphate-buffered saline (PBS) at pH 7.4. Prior to ICP-MS measurement, the pellet was resuspended in PBS buffer to make sure cell density of $10^6$ $mL^{-1}$. The number of cells was counted by haemocytometer under an optical microscope. The cell suspension was sprayed into aerosols using microconcentric nebulizer and introduced into the ICP directly for time-resolved ICP-MS measurements.

### 3.2 Time-resolved ICP-MS measurement

The experimental details of the single cell analysis by time-resolved ICP-MS have been described previously.[1] Briefly, the sample (leukemia cell suspension) was placed in a 500-μL syringe for injection into the nebulizer. A micro-syringe pump (SPLab01. Baoding Shenchen precision pump Co., Ltd., Hebei, China) was used to precisely control the flow rate at 0.03 mL/min. A microconcentric nebulizer and a single-pass spray chamber were used for aerosol generation. During each integration time (5 ms) at most one cell is allowed to be analyzed after the sampling rate and integration time being controlled precisely. Only one isotope was monitored in each measurement. For $^{75}As$ detection, helium gas mode was applied, for other metals ($^{195}Pt$, $^{146}Nd$, $^{163}Dy$) detection, no gas mode was used. Before each experiment, the ICP-MS was tuned using an aqueous multi-element standard solution (1 ppb each of Li, Y, Co, Ce and Tl) for consistent sensitivity ($^{7}Li$, $^{89}Y$ and $^{205}Tl$) and minimum levels of doubly-charged ions and oxide species of $^{140}Ce$. The measurement duration was typically 60 sec. All measurements were triplicated. Data were analyzed offline using Origin (Origin version 9) and Microsoft Excel (Microsoft Excel 2013).

### 3.3 Cytotoxicity of $As_2O_3$ treated leukemia cells

Sample cells were harvested after treatment with a serials concentration of $As_2O_3$ for 24 hrs. About $10^6$ $As_2O_3$ treated leukemia cells were stained with 25 μM cisplatin in serum-free RPMI for 1 min at room temperature. The reaction was quenched with 3 ml of RPMI/10% FBS. Cell suspension was removed by 3 repetitions of centrifugation (1000×g, 5 min) and cell pellets were resuspended in PBS and processed for time-resolved ICP-MS assay.

### 3.4 Apoptosis of $As_2O_3$ treated leukemia cells

About $10^6$ cells were stained with 1 μg/ml Annexin V-DOTA-Nd conjugates in binding buffer (10 mM Hepes at pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) for 30 min at room temperature. Cell suspension was removed by 3 repetitions of centrifugation (1000×g, 5 min) with PBS, cell pellets were resuspended in PBS and processed for time-resolved ICP-MS assay.

About $10^6$ cells were collected by 3 repetitions of centrifugation with PBS. Cells were resuspended in 500 μL of 1X binding buffer, 5 μL of Annexin V-FITC and 10 μL of propidium iodide (PI) were added, and then incubated at room temperature for 15 min in the dark prior to flow cytometry analysis.

### 3.5 Differentiation of $As_2O_3$ treated leukemia cells

About $10^6$ leukemia cells treated by $As_2O_3$ were firstly blocked with 5% BSA for 30 min at room temperature and then stained with 5 μM CD11b-DOTA-Dy conjugates for 30 min at room temperature. Cell suspension was removed by 3 repetitions of centrifugation (1000×g and 5 min), cell pellets were resuspended in PBS and processed for time-resolved ICP-MS assay.

Samples were firstly blocked with 5% BSA for 30 min at room temperature and then incubated with 5 μM monoclonal anti-CD11b−FITC at 4°C for 30 min in the dark. The cells were washed three times with PBS and resuspended in 500 μL of PBS with 0.5% BSA for flow cytometry analysis.

**Table S1.** Operating conditions for $^{75}As$ detection at a single cell level

| | |
|---|---|
| RF power (W) | 1550 |
| RF Matching (V) | 1.80 |
| Sampling Depth (mm) | 6.0 |
| Carrier Gas (L/min) | 0.8 |
| S/C Temp (°C) | 2 |
| Makeup Gas (L/min) | 0.25 |
| Extract 1 (V) | 0 |
| Extract 1 (V) | -165 |
| Omega Bias (V) | -80 |
| Omega Lens (V) | 7.9 |
| Cell Entrance (V) | -30 |
| Cell Exit (V) | -50 |
| Deflect (V) | 12.0 |
| Plate Bias (V) | -40 |
| He Gas (mL/min) | 0.7 |
| OctP Bias (V) | -8.0 |
| OctP RF (V) | 160 |
| Energy Discrimination (V) | 4.5 |
| Total uptake rate (mL/min) | 0.03 |
| Integration time (s) | 0.005 |
| Measurement duration (s) | 60 |

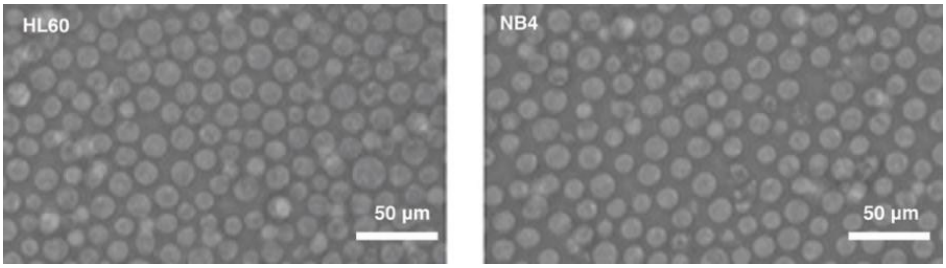


**Figure S1**. Microscopic images of HL60 and NB4 cells in culture medium. Both HL60 and NB4 are monodispersed and maintain their morphological integrity in culture medium.

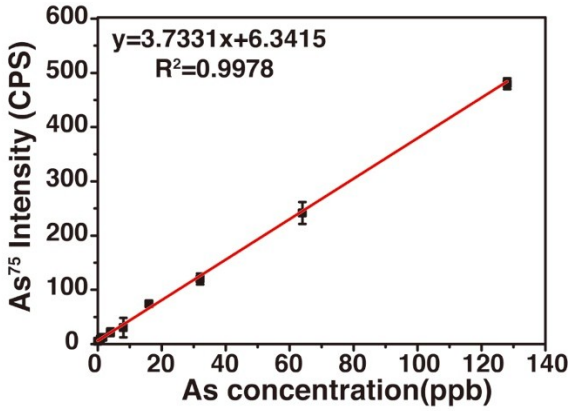


**Figure S2**. $^{75}$As calibration curve using standard arsenic solutions by time-resolved ICP-MS.

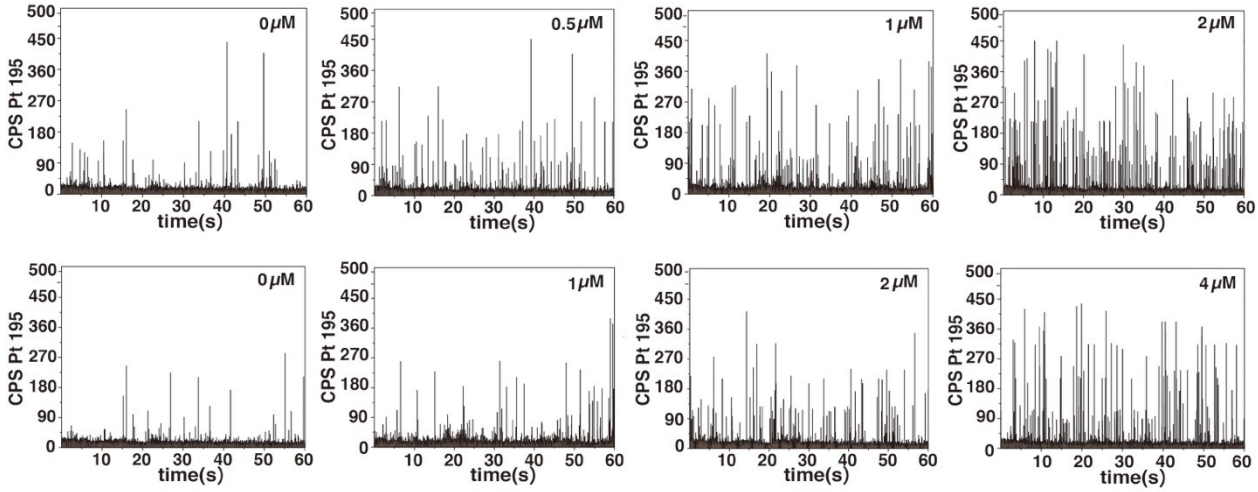


**Figure S3**. $^{195}Pt$ profiles of $As_2O_3$ treated NB4 (Top) and HL60 (Bottom) cells by time-resolved ICP-MS.

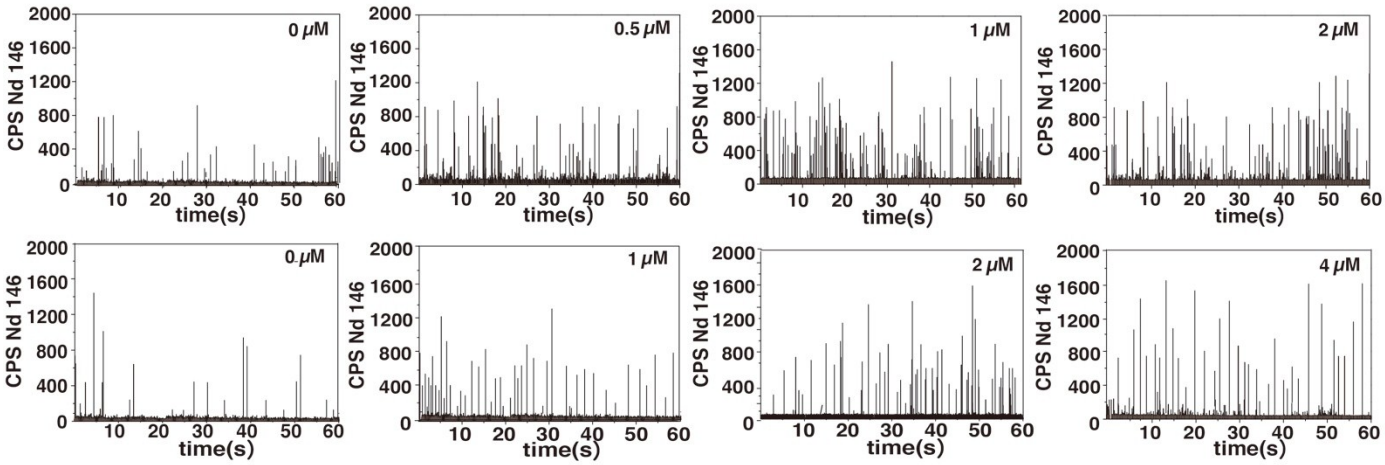


**Figure S4**. $^{146}Nd$ profiles of $As_2O_3$ treated NB4 (Top) and HL60 (Bottom) cells by time-resolved ICP-MS.

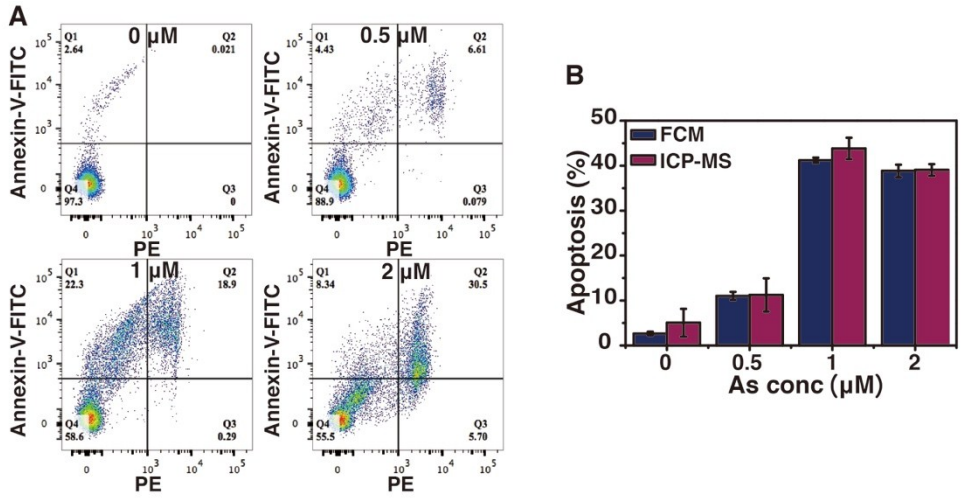


**Figure S5**. (A) Apoptosis of NB4 cells upon treatment with different concentrations of $As_2O_3$ for 24 hrs by flow cytometry; (B) Comparison of amounts of cell apoptosis detected by flow cytometry with those by time-resolved ICP-MS. A very consistent result indicates the feasibility of using time-resolved ICP-MS in investigation of cell apoptosis.

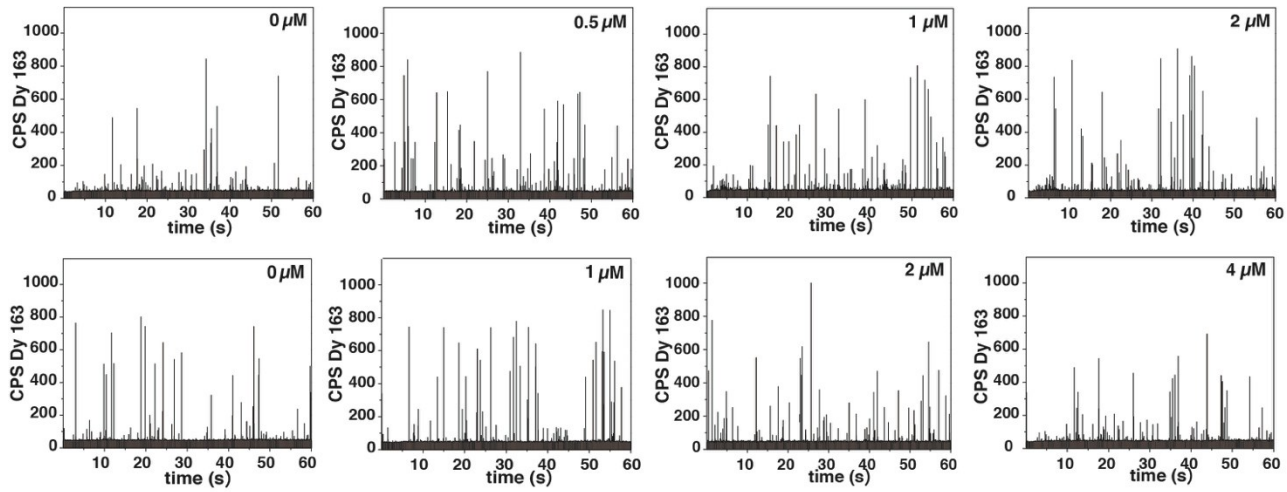


**Figure S6**. $^{163}$Dy profiles of $As_2O_3$ treated NB4 (Top) and HL60 (Bottom) cells by time-resolved ICP-MS.

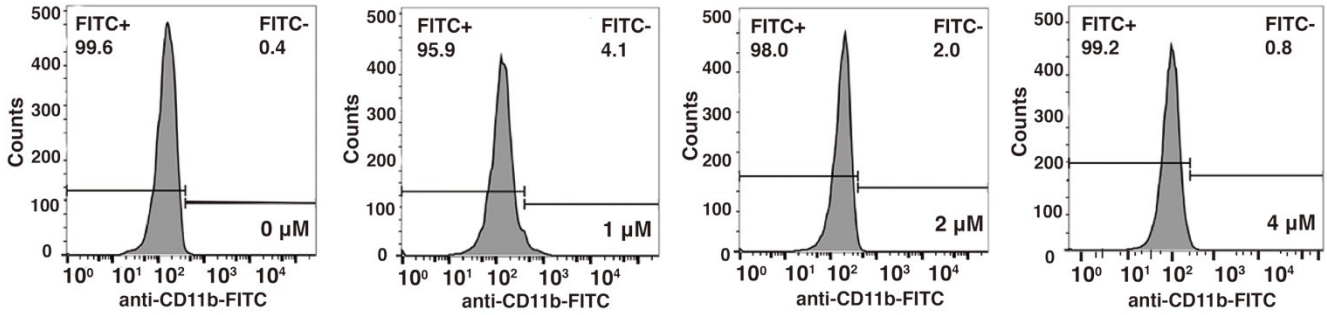


**Figure S7**. Differentiation of HL60 cells after treatment with different amounts of $As_2O_3$ for 24 hrs by flow cytometry. No obvious differentiation was shown in $As_2O_3$ treated HL60 cells.

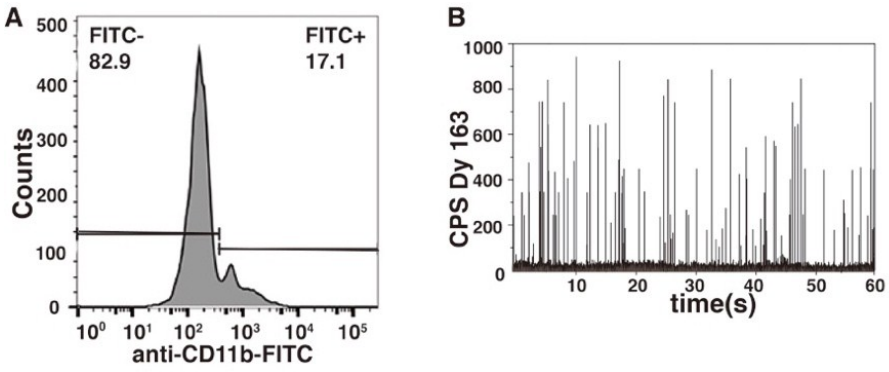


**Figure S8**. Differentiation of HL60 cells treated with 1 μM ATRA for 24 hrs by flow cytometry (A) and time-resolved ICP-MS (B). It is noted that ATRA induces significant cell differentiation, different from $As_2O_3$.

**Reference**

1. C. N. Tsang, K. S. Ho, H. Sun, W. T. Chan, *J. Am. Chem. Soc.* 2011, **133**, 7355-7357.