Antitumor Lymphocyte Kinetic Cytotoxicity Assay

In collaboration with Nikon, the Centre for Commercialization of Regenerative Medicine ("CCRM") has developed a **kinetic cytotoxicity assay to measure the potency of antitumor lymphocytes,** which may be of interest to industry members **involved in anticancer immunotherapy (e.g. NK- and T-cells).**

1 | Market Need

Adoptive transfer of antitumor lymphocytes has received significant attention from investigators in the field of cancer therapeutics. A key element to characterisation of immunotherapeutic lymphocytes is quantification of their ability to destroy cancer cells. Cytotoxicity is traditionally measured either by a ^{51}Cr-release or flow cytometry assay after incubation with a human leukemia cell line (K562 or Jurkat). The ^{51}Cr-release assay is limited by its expense as well as safety concerns around the use of radioactivity. In addition, neither ^{51}Cr-release nor flow cytometry provides kinetic information on potency. One source of lymphocytes for anticancer immunotherapy is NK-92 cells, a human natural killer (NK) cell line that displays cytotoxic activity towards a variety of cancer derived cell types. NK cell-based adoptive immunotherapies have been used in Phase I trials to treat renal cancer and melanoma (USA), colon/lung cancer (Germany), acute myeloid leukemia, AML (USA and Canada) and acute lymphoblastic leukemia, ALL (Canada). Working with the NK-92 cell line, CCRM has developed a kinetic potency assay using the BioStation CT incubated fluorescence imaging system.

2 | Products

- A kinetic cytotoxicity assay for antitumor lymphocytes (e.g. NK- and T-cell-based)

3 | Competitive Advantages

- Provides real-time kinetic data on cytotoxicity
- Allows for observation of cell-cell interactions in real-time
- Not reliant on radiactive material
- Comparable to flow-cytometry based analysis (**Figure 1**)

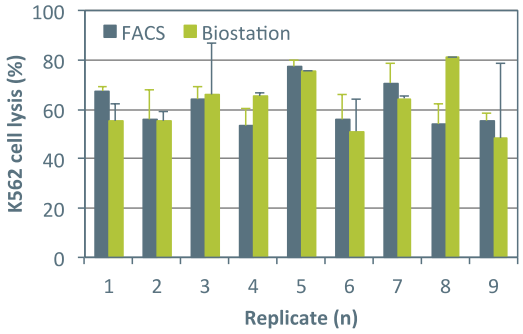

Figure 1. BioStation CT shows good correlation in values of target cell lysis obtained with flow-cytometry.

4 | Technology

4.1 Technology Description

The Nikon BioStation CT® is a compact cell incubator and monitoring system that allows users to conduct live cell imaging and facilities a broad array of long-term time-lapse experiments, including studies of cell growth, morphology and protein expression. It provides consistent environmental control of temperature, humidity and gas concentration in combination with phase contrast and fluorescence imaging. The kinetic potency assay utilises the Nikon BioStation CT® and BS-FL fluorescence unit for time-lapse imaging of fluorescently labelled target cell destruction by anti-tumour lymphocytes. An associated Nikon CL-Quant algorithm quantifies the decrease in fluorescence intensity due to target cell death over a four-hour co-incubation with effector cells.

4.2 Proof of Principle

The cytotoxicity assay in the BioStation CT assay was performed as follows:

1. K562 target cells were stained with Calcein-AM viability dye.
2. Target cells (T) were then incubated with NK-92 effector cells (E) at a ratio of E:T of 10:1 for four hours in the BioStation CT, in 96-well plate format.

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Fluorescence intensity was monitored and target cell death was measured by loss in calcein fluorescence. Spontaneous target cell death was also monitored during this time and used as the control. K562 cells incubated with NK-92 cells for four hours show 44 ± 4% lysis (**Figure 2A-B**), with 4 ± 0.6% accounting for spontaneous cell death (**Figure 2D**). Kinetic data is also generated using the Biostation CT® and is shown in **Figure 2C**. Furthermore, microscopy images (**Figure 2A-B**) show cell dynamics, i.e. effector cells form clusters surrounding target cells within the first two hours of incubation.

Data obtained by the BioStation CT was compared to a flow cytometry method based on Kim *et al.,* 2007 and Thakur *et al.*, 2012. Cell tracker orange (CTO)-labelled K562 target cells were incubated with NK-92 effector cells at an optimised E:T ratio of 10:1 and stained with the Calcein-AM viability dye prior to analysis. Lysis of CTO-labelled K562 cells was quantified by the increase in the CTO+/calcein- population. **Figure 1** shows that the percentage of K562 lysis obtained with the BioStation CT is comparable to the flow cytometry assay, and is therefore an effective alternative tool to assess NK-92 cell cytotoxicity *in vitro* and in real-time.

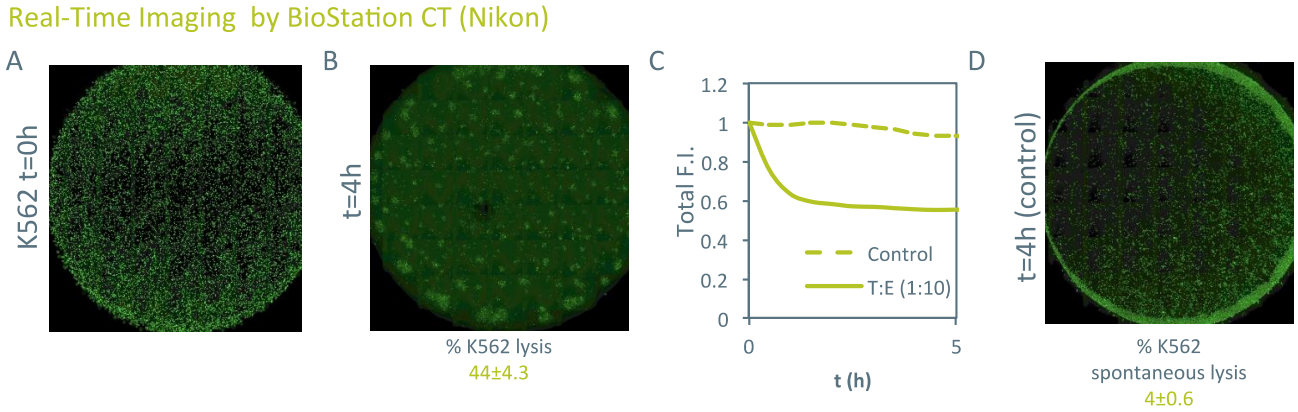

Figure 2. Real-time imaging for the assessment of NK-92 cell cytotoxicity using the BioStation CT. (A) Fluorescent image of K562 cells stained with calcein at t=0 hr (B) Fluorescent image of K562 lysis by action of NK-92 cells after 4 hr (C) Kinetic plot of the decay in fluorescence intensity (F.I.) measured using BioStation CT and CL-Quant software (Nikon). (D) Fluorescent image of the spontaneous lysis after 4 hr.

4.3 Intellectual Property

- No IP has been filed for this assay. Nikon owns the rights to the BioStation CT®.

4.4 Lead Inventor(s)

- Dr. Nick Timmins and Dr. Ricardo Baptista at the Centre for Commercialization of Regenerative Medicine, Toronto, Ontario
- Nikon

4.5 Relevant Reference

Kim et al (2007). *J. Immunol Methods*, 325 (1-2): 51-66. PMID17617419
Thakur *et al.*, (2012) *Biotechnol Lett*, 34(3): 447-53. PMID: 22187077

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