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Analyzing immune cell infiltration of cancer spheroids in a 3D cell culture platform / Hien Lim, Sei; Mun Kuan, Chee; Campisi, Marco; Chiono, Valeria; Pavesi, Andrea. - In: CANCER RESEARCH. - ISSN 0008-5472. - 79:13(2019), pp. 47-47. [10.1158/1538-7445.AM2019-47]

*Availability:*

This version is available at: 11583/2852591 since: 2020-11-13T01:57:15Z

*Publisher:*

American Association for Cancer research

*Published*

DOI:10.1158/1538-7445.AM2019-47

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**Control/Tracking Number:** 19-A-4587-AACR

**Activity:** Abstract Submission

**Current Date/Time:** 11/15/2018 9:21:58 PM

**Analyzing immune cell infiltration of cancer spheroids in a 3D cell culture platform**

**Short Title:**

3D immune cell infiltration assay

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**Abstract:**

The adoption of *in vitro* 3D cell culture models as a bridge between conventional 2D cell culture models and the complex *in vivo* animal models has been increasing. A carefully designed 3D model can run biological assays with animal model-like complexities but with the simplicity and affordability of traditional cell culture. For example, traditional 2D and transwell assays of immune cell infiltration efficiency are unrealistic, as cell migration is gravity-driven. AIM 3D cell culture chips offer a more realistic immune cell infiltration model by compartmentalizing immune cells and cancer spheroids in parallel channels. The chips consist of a 3D hydrogel channel and two flanking media channels. Cancer spheroids are cultured within the hydrogel channel while immune cells are seeded in one of the media channels. The seeded immune cells are required to actively invade and seek out target cancer cells in 3D hydrogel in AIM chips before they can infiltrate the cancer spheroids. This is a process similar to immune cell infiltration *in vivo*. By utilizing high content confocal imaging, fluorescent-labeled immune cells that migrate into the 3D hydrogel can be visualized and quantified. This assay, in combination with adoptive T cell therapy or immune checkpoint blockade, can determine the roles of tumor infiltrated lymphocytes in immunotherapy through quantifying the live: dead ratio of cancer spheroids in the chips. This is particularly useful as a quality control tool for cellular adoptive immunotherapy where the infiltration efficiency and tumoricidal activity of engineered immune cells can be accessed *in vitro*. In summary, AIM 3D cell culture chips create a more physiologically relevant 3D microenvironment for visualizing immune cell infiltration of cancer spheroids.

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Author Disclosure Information:

**S. Lim** ; AIM Biotech Pte Ltd. **C. Kuan** ; AIM Biotech Pte Ltd. **M. Campisi** ; MIT-POLITO grant (BIOMODE - Compagnia di San Paolo) under the joint doctorate with University of Turin; ph.D scholarship. **V. Chiono** ; MIT-POLITO grant (BIOMODE - Compagnia di San Paolo) under the joint doctorate with University of Turin; Supervisor. **A. Pavesi** ; AIM Biotech Pte Ltd.

**Sponsor (Complete):**

**Category and Subclass (Complete):** TB01-01 3-D and tissue recombinant models

**Research Type (Complete):** Basic research

**Organ Site/Structures (Complete):**

\***Primary Organ Site:** Not Applicable

\***Choose Chemical Structure Disclosure Option:**

NOT APPLICABLE. No compounds with defined chemical structures were used.

\***Please explain reason for not disclosing (maximum 250 characters with spaces):** : NA

\***Reference or patent application number** : NA

**Keywords/Indexing (Complete):** 3D models ; Immuno-oncology ; Adoptive immunotherapy ; Phenotypic assays

**Financial Support for Attendance (Complete):**

**Submission Fee (Complete):** Your credit card order has been processed on Thursday 15 November 2018 at 9:18 PM.

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