Monitoring the Oncolytic Effect of Talimogene laherparepvec (T-VEC) in Combination With the MEK Inhibitor Trametinib Using Laser Force Cytology

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Background 1

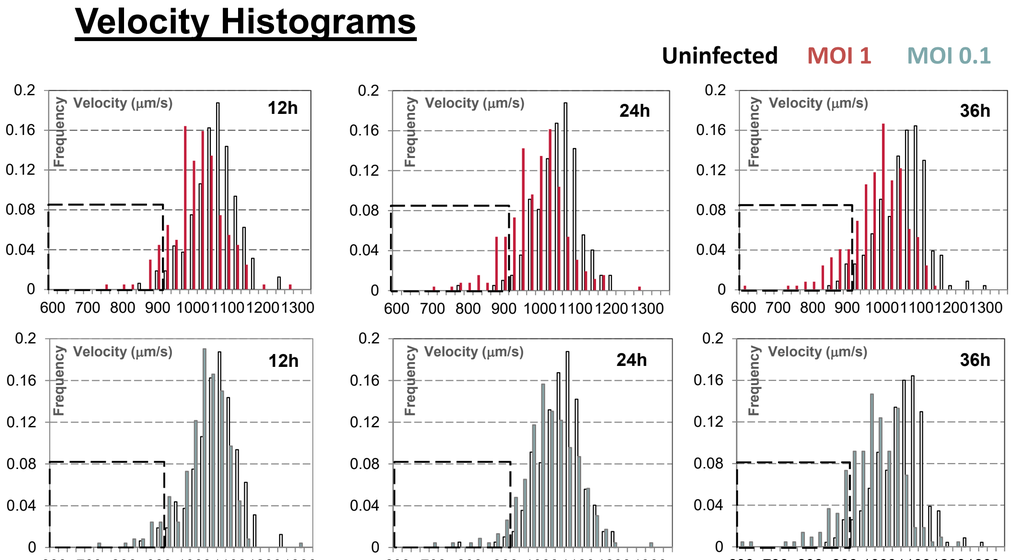
Introduction

The ability to rapidly and accurately determine the viral infectivity and oncolytic effects of drug candidates could help improve the speed of oncolytic vaccine R&D, product development, manufacturing, and efficacy monitoring. Here we present the results from monitoring the drug Talimogene laherparepvec (T-VEC) infection in SK-MEL-28 melanoma cancer cells using Laser Force Cytology (LFC). SK-MEL-28 cells are tumorigenic human cells derived from a 51 year old male malignant melanoma cancer patient. Metastatic melanoma does not respond well to chemotherapy or radiation and therefore is a prime candidate for oncolytic viral therapy. Using a combination of multiple variables measured by LumaCyte's Radiance™ instrument, an infection metric was developed that correlates well with the initial virus concentration, demonstrating the potential for rapid infectivity and oncolytic measurements using LFC.

Materials and Methods

SK-MEL-28 cells (ATCC HTB-72) were grown in RPMI with 5% FBS and seeded into 24-well plates. After 24 incubation at 37° C and 5% CO_2, the medium was aspirated from each well and replaced with RPMI without FBS for 12h before addition of 10nM Trametinib (if indicated) for a period of 12h. Subsequently, cells were infected with TVEC at the indicated MOI. The medium was aspirated and replaced with 199V medium containing the virus or an equivalent volume of medium only. The cells were then placed in the incubator for 1 hour before overlaying with an equal volume (250 μL) of RPMI with or without Trametinib. Cells were harvested at the indicated timepoints and analyzed using a Radiance instrument.

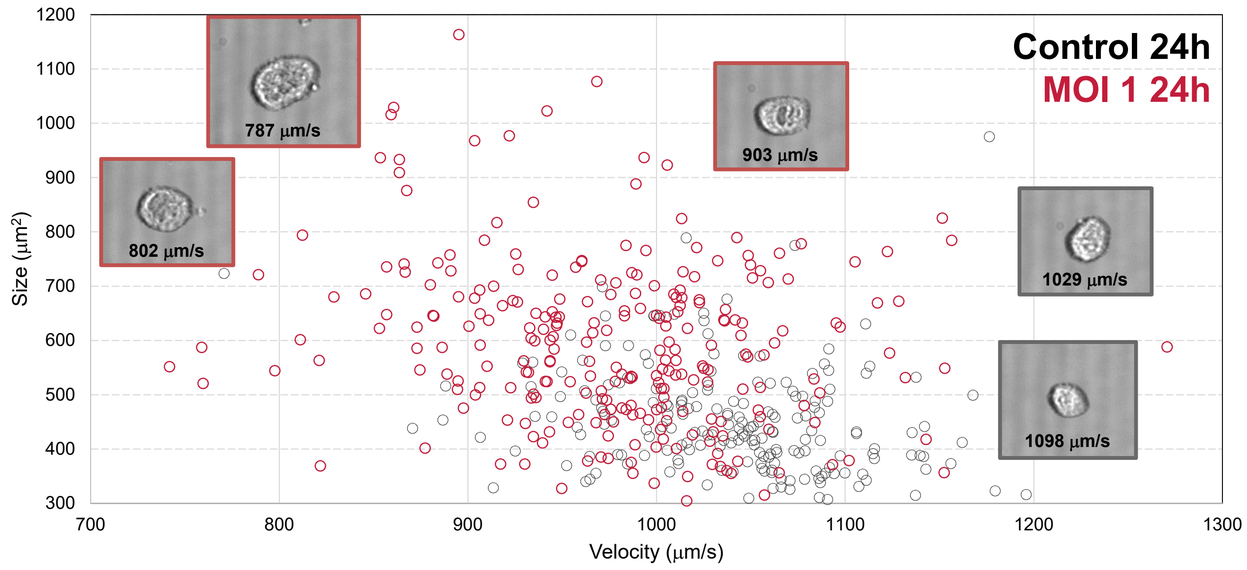
Velocity Histograms



Velocity histograms comparing SK-MEL-28 cells infected with T-VEC to uninfected cells at MOI 1 or 0.1 sampled at 12, 24, and 36 hours post infection. Frequency represents the percentage of the cell population with a velocity that fits into a particular bin. The dotted box encloses cells with a velocity below 900 μm/s, and is used to calculate the infection metric.

Monitoring T-VEC Infection 2

Size vs Velocity Scatter Plot and Cell Images

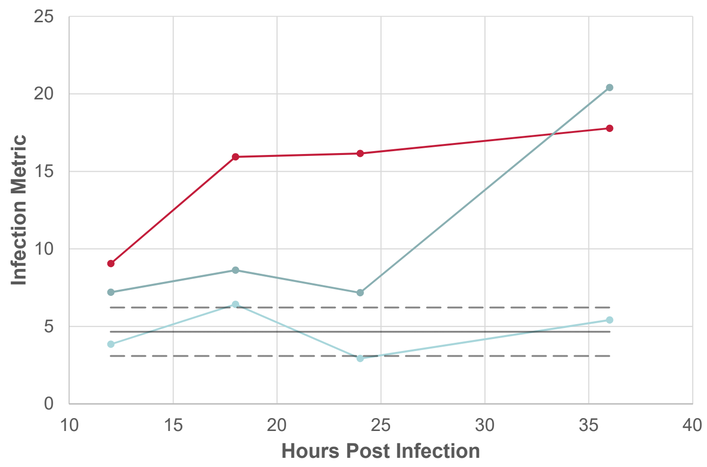


Velocity: A measure of how fast the cell travels through the laser interrogation region. It is related to the cell's optical force: a lower velocity indicates a higher optical force and vice versa.

Size vs Velocity scatter plot comparing SK-MEL-28 cells infected with T-VEC to uninfected control cells at 24 hours post infection. Each point on the graph represents one cell, representative images of which are shown on the graph. The number associated with each image indicated its velocity value.

Biophysical changes occur upon T-VEC infection

- Increase in size and decrease in velocity can be used to track progress of the infection



Infection Metric = % of cells below a threshold velocity

- Cells infected at MOI 1 change little after 18h, while cells infected at MOI 0.1 increase through 36h

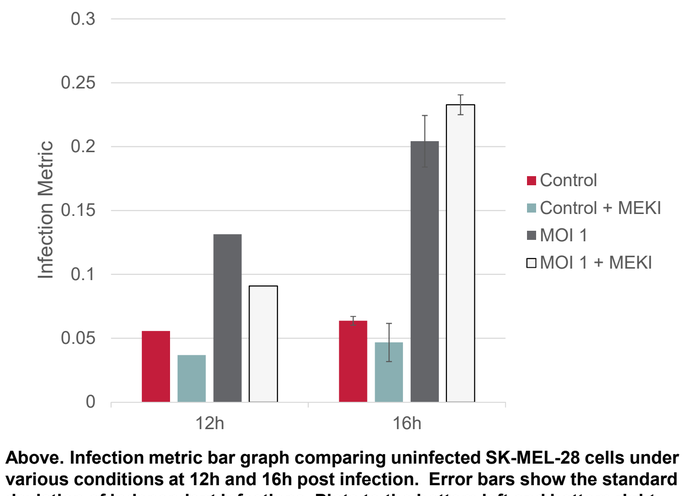
Plot comparing changes in the infection metric over time for SK-MEL-28 cells infected with T-VEC at MOI 1.0 and MOI 0.1 to uninfected control cells. The solid and dashed lines represent the mean and standard deviation of uninfected cells throughout the time course.

Effects of MEKI on T-VEC Infection in SK-MEL-28 Cells 3

Radiance data illustrates how the MEKI alters the infection process

Benefits: Rapid assessment of known and potentially unknown phenotypic changes

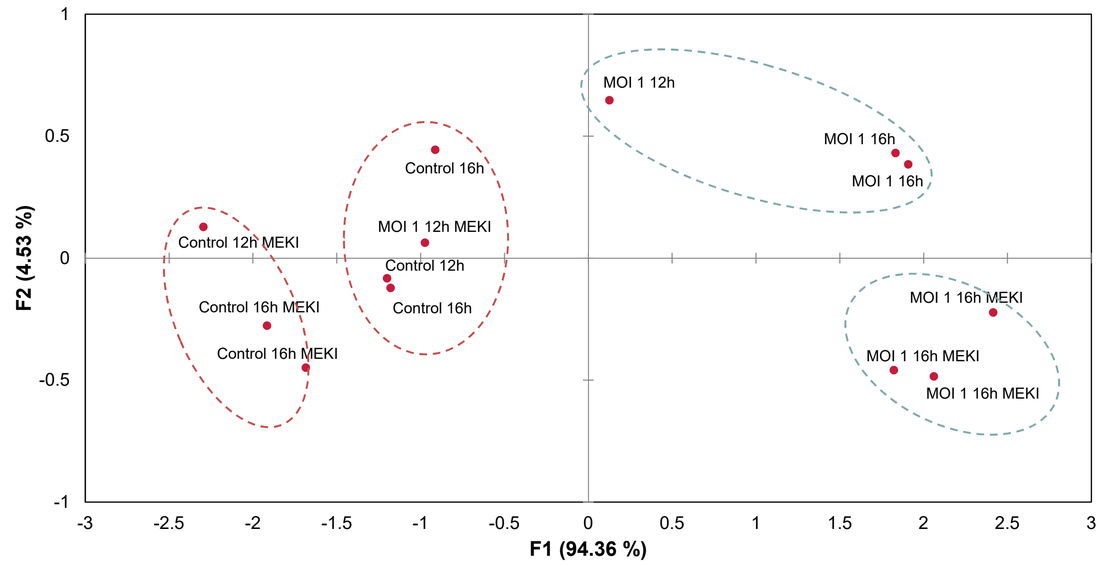
Infection Metric



- Infection Metric = % of cells below a threshold velocity
- At 12h, the addition of the MEKI decreases the infection metric for both uninfected and infected cells
- At 16h, the trend is reversed for the infected cells, where the cells plus MEKI show an increase in infection metric

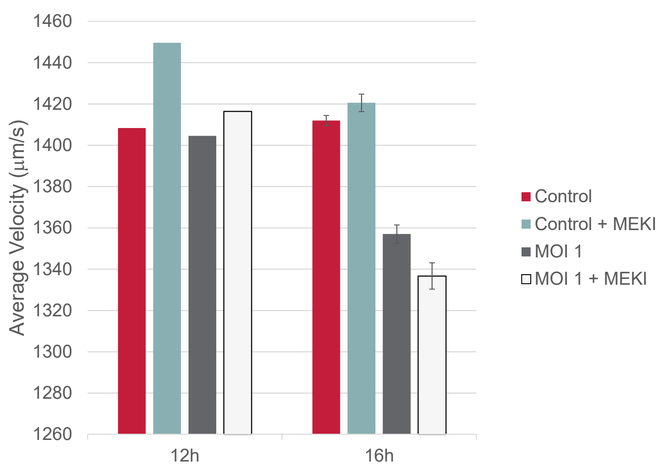
Above. Infection metric bar graph comparing uninfected SK-MEL-28 cells under various conditions at 12h and 16h post infection. Error bars show the standard deviation of independent infections. Plots to the bottom left and bottom right show the Velocity and Size of the same cell populations.

Principal Component Analysis



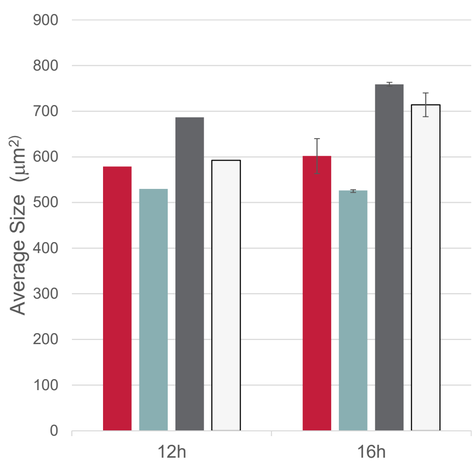
Principal component analysis using infection metric, average velocity, and average size. Each point represents an independent sample population at the indicated condition.
Note – circles are not statistically derived and are for qualitative purposes only

Average Velocity



- At 12h, average velocity does not show the change that infection metric does
- Illustrates the detectable change in a small subpopulation of cells that might be missed when only looking at the average velocity

Average Size



- Infection with T-VEC increases the size of the cells
- Addition of the MEKI decreases the size of the cells

- Using Principal Component Analysis (PCA), two factors (F1 and F2) were developed that account for >98% of the variation in the data
- With the exception of MOI 1 12h + MEKI, uninfected samples group on the left (F1 < 0), while infected samples group on the right (F1>0)
- Among the infected samples to the right, samples with and without MEKI group on the bottom (F2 < 0) and top (F2>0) quadrants, respectively
- Multivariate analysis techniques such as PCA highlight the flexibility and utility offered by Laser Force Cytology to analyze cells based on label-free biophysical parameters

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