

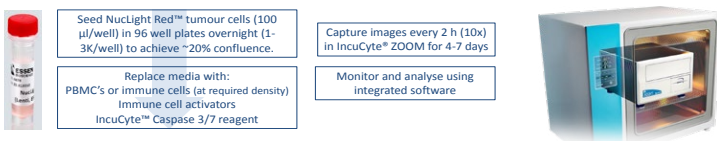
Novel Kinetic Live Cell Imaging Assays for Immune Cell Killing of Tumor Cells

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Summary and Impact

- Cancer immunotherapy offers the potential for specific eradication of tumour cells and the prevention of cancer recurrence.
- Here we describe fully kinetic live cell imaging assays to quantify immune cell mediated killing of adherent and suspension tumour cells in co-culture.
- RFP-nuclear labelled tumour cells were seeded either overnight in 96 well plates or at time of assay in ultra-low attachment U-bottom plates. Immune cells (PBMCs, isolated CD8⁺ T or NK cells) were then added to the wells in combination with various activators and a non-perturbing caspase 3/7 apoptosis green fluorescence detection reagent.
- Phase and dual-colour fluorescence images were captured every 2h for 4-7 days using IncuCyte® ZOOM.
- Tumour cell numbers were quantified by direct nuclear object counting (RFP). The number of apoptotic tumour cells were measured using green (caspase 3/7) object counting and size/brightness filtering to remove dying immune cells.
- This method is illustrated and validated with anti-CD3 and IL-2 mediated cell killing and antibody driven cell killing both with adherent and suspension target cells.
- These validation data demonstrate that live cell imaging can be used to discern the full time course and specificity of immune cell killing, without the need to lift cells, use Ab labels or radioisotopes (e.g. Cr⁵¹).
- HD images and time-lapse movies facilitate a clear understanding & compelling verification of the underlying biology.

Effector/Target Cell Co-Culture Assay Workflow



- To enable direct analysis of the target tumour cell proliferation, various target tumour cell lines were created using nuclear restricted RFP (NuLight Red™ cell lines, Essen BioScience).
- IncuCyte™ Caspase 3/7 Reagent, a non perturbing DEVD- substrate linked fluorogenic probe is used to label apoptotic cells (green fluorescence).
- Method suitable for use with 384 well plates.

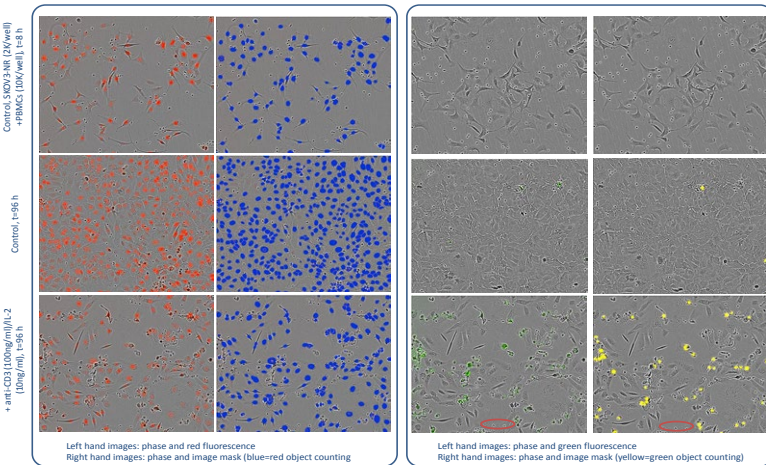
Quantification of Tumor Cell Proliferation and Apoptosis

Proliferation

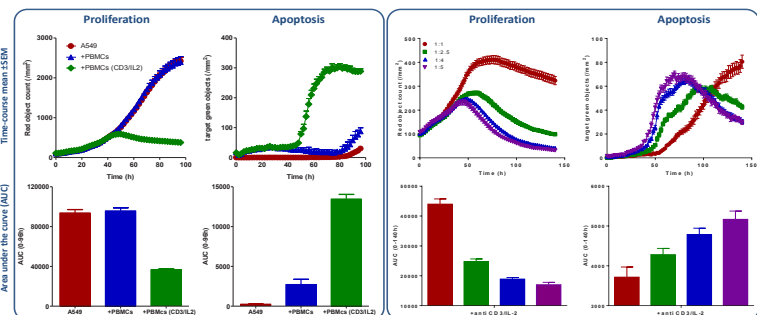
- Tumour cell number was quantified by image-masking and counting the number of red objects (nuclei).
- Note that T cell activation (anti-CD3/IL-2) markedly attenuates the number of tumour cells in the co-culture after 96 h.

Apoptosis

- Tumour cell apoptosis was quantified by image masking and counting the number of green-labelled nuclei (caspase 3/7 +ve). Analysis filters were applied to remove small, spherical green objects (red ring) that represent apoptotic PBMCs.
- Note the marked increase in apoptotic tumour cells following T cell activation.

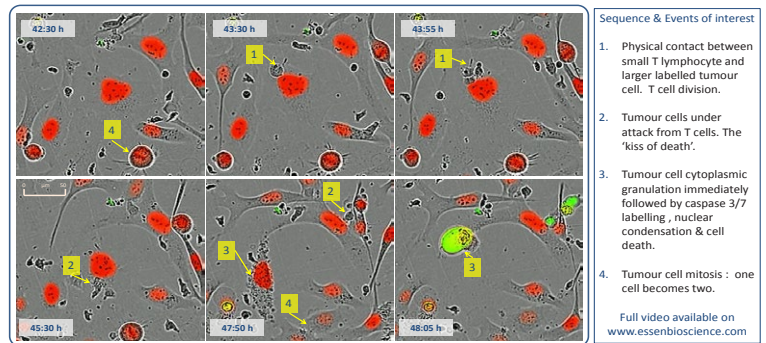


Time-Courses of Growth Arrest and Immune Cell Mediated Apoptosis



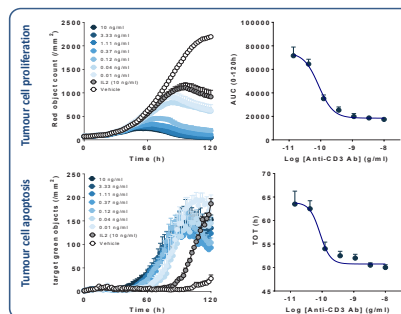
- Data shown are A549 NuLight Red™ cells (2K/well) in combination with PBMCs (20K/well), with activation of T cell population with IL-2 and anti-CD3 activation.
- Note the delayed onset (40 h) of T cell mediated apoptosis and growth arrest of tumour cells following immune cell activation.
- Data shown are SKOV3 NuLight Red™ cells (2K/well) in combination with increasing numbers of PBMCs (2, 5, 8, 10K/well) with activation.
- Increasing PBMC:tumour cell increases tumour cell apoptosis and growth arrest signal and decreases the onset time.

Time-Lapse Images Verify Underlying Biology



- SKOV3 NuLight Red™ cells (2K/well) seeded with PBMCs (20K/well) with activation by anti-CD3 and IL-2 in the presence of caspase 3/7 reagent. The images were captured every 5 mins using a 20x objective.

Pharmacology of Anti-CD3 Mediated Tumor Cell Killing

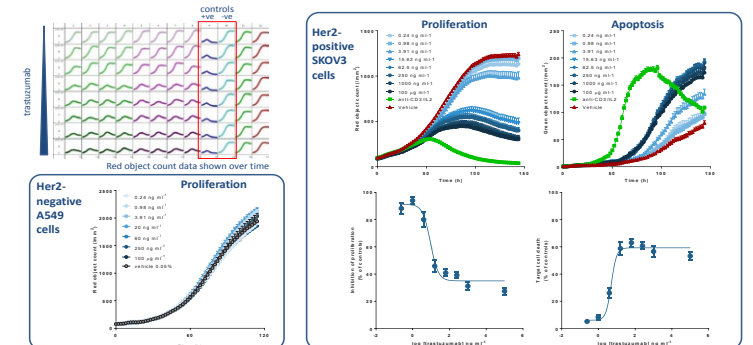


- A549 NuLight Red™ cells (2K/well) were seeded with human CD8⁺ T cells and activated with increasing concentrations of anti-CD3 antibody (0.01-10 ng/ml) in the presence of IL-2 (10 ng/ml).

- Data demonstrates a concentration dependent decrease in target tumour cell proliferation with an IC₅₀ of 85.3 pg/ml (AUC).

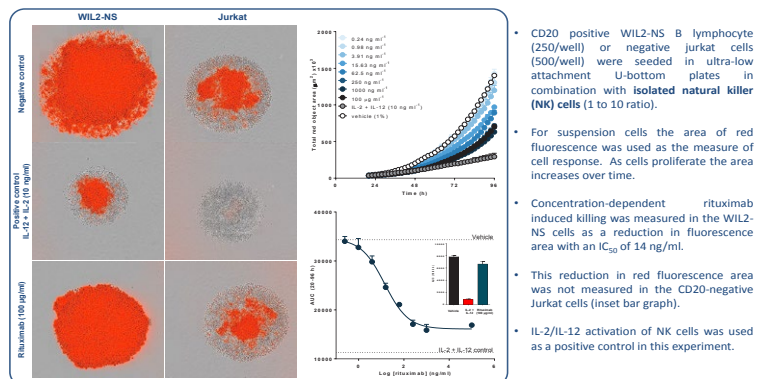
- The data shows a reduction in the time taken to induce target tumour cell apoptosis with an apoptosis threshold onset time mid point of 85.4 pg/ml.

Trastuzumab Induced ADCC in Her2 Positive SKOV3 Cells



- Her2 positive SKOV3 or negative A549 NuLight Red™ cells (1.6K/well) in combination with PBMCs (8K/well) were tested in the presence of trastuzumab to induce antibody-dependent cell-mediated cytotoxicity (ADCC).
- A concentration-dependent decrease in proliferation (IC₅₀ 8.1 ng/ml) and increase in apoptosis (4.6 ng/ml) was measured in Her2-positive SKOV3 cells.
- No response was seen in Her2-negative A549 cells.

Rituximab Induced Killing of CD20 Positive WIL2-NS Cells



- CD20 positive WIL2-NS B lymphocyte (250/well) or negative Jurkat cells (500/well) were seeded in ultra-low attachment U-bottom plates in combination with isolated natural killer (NK) cells (1 to 10 ratio).

- For suspension cells the area of red fluorescence was used as the measure of cell response. As cells proliferate the area increases over time.

- Concentration-dependent rituximab induced killing was measured in the WIL2-NS cells as a reduction in fluorescence area with an IC₅₀ of 14 ng/ml.

- This reduction in red fluorescence area was not measured in the CD20-negative Jurkat cells (inset bar graph).

- IL-2/IL-12 activation of NK cells was used as a positive control in this experiment.