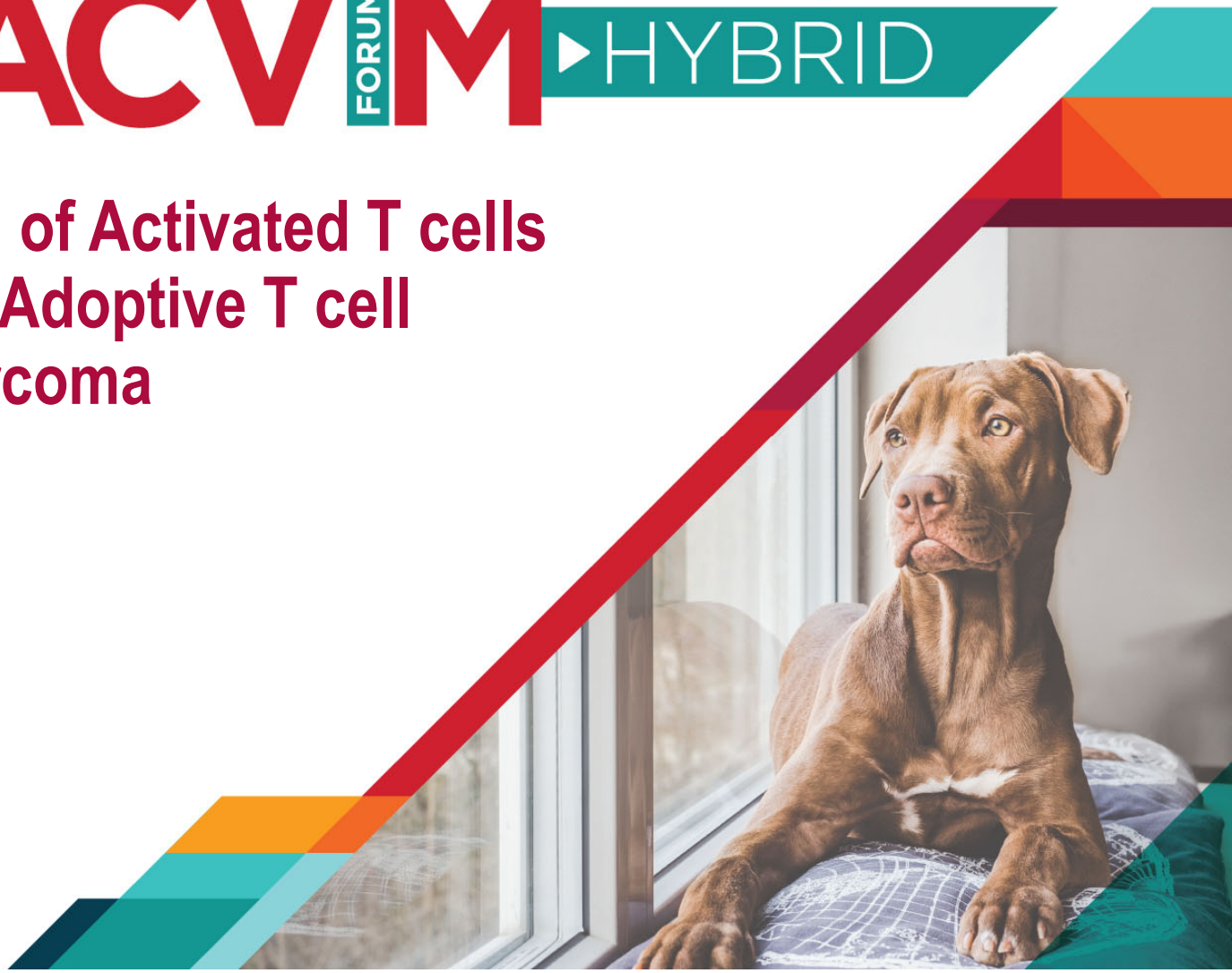
2022 ACVIM FORUM HYBRID

Cytokine Expression of Activated T cells in Dogs Undergoing Adoptive T cell therapy for Osteosarcoma

Noe Reyes, DVM

June 24, 2022 | 9:15 AM



RECONNECT
to Community, Science + Medicine

Speaker Disclosure

Cytokine Expression of Activated T cells in Dogs Undergoing Adoptive T cell therapy for Osteosarcoma

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FINAL DISCLOSURE:

I am the Chief Medical Officer for ELIAS Animal Health.

UNLABELED/UNAPPROVED USES DISCLOSURE:

I will discuss data from a clinical trial for a vaccine enhanced adoptive T cell immunotherapy that is currently not approved for use in animals.

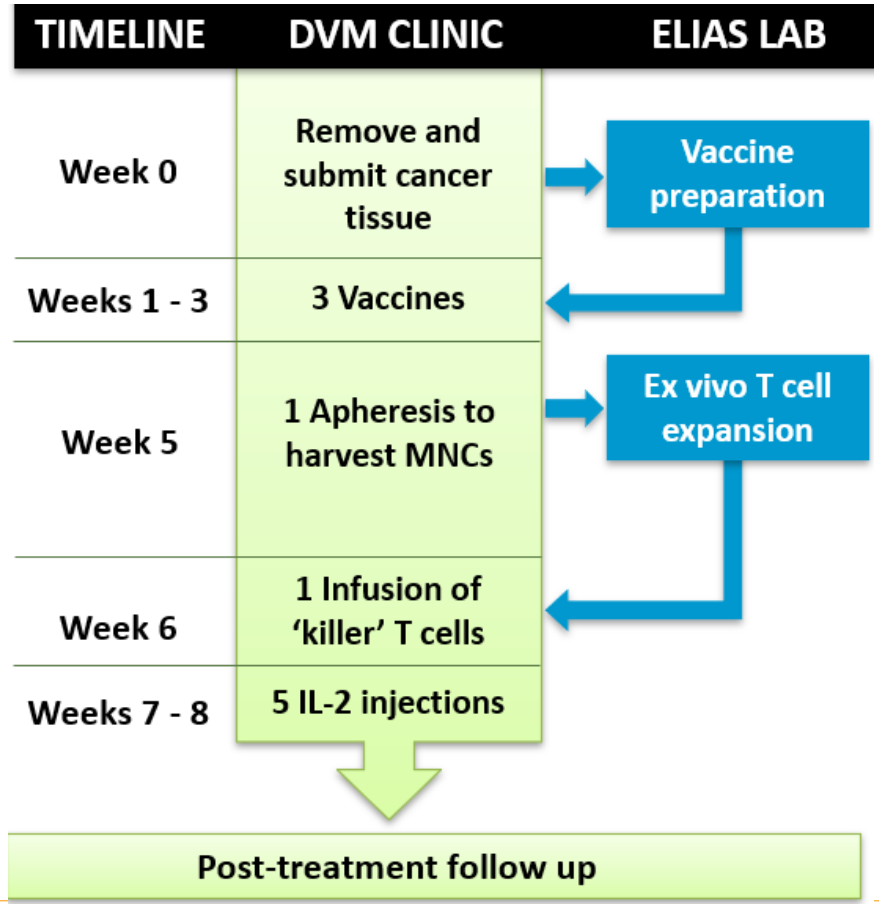
Study Overview

- Purpose of study was to confirm the mechanism of action for “vaccine-primed adoptive T cell” immunotherapy (VACT) in canines
- VACT therapy uses autologous cancer cell vaccines to condition immune cells to the host tumor-specific cancer antigens
- The VACT protocol has previously demonstrated efficacy in humans (Sloan, 2000, Chang, 2003) and in canine osteosarcoma (Flesner, 2020)
- Cytotoxicity and cytokine expression of immune cell from samples examined at 3 different time points during the protocol for each patient

Study conducted at Charles River Discovery Research Services, Freiburg, Germany

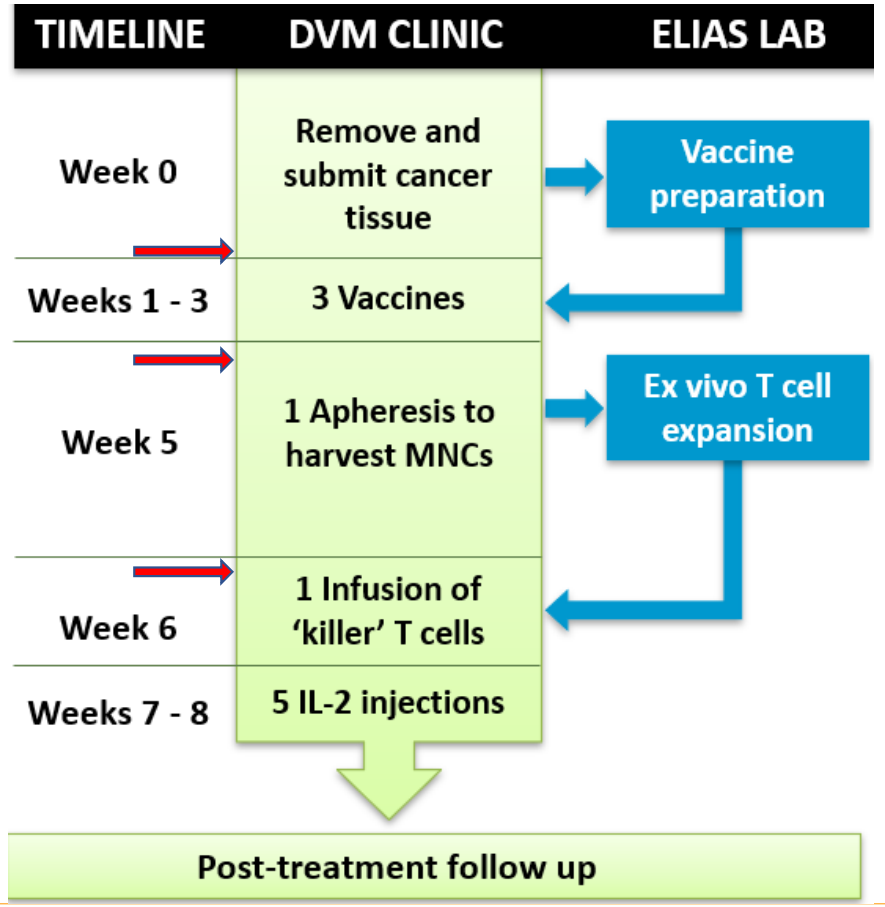
Overview of VACT - Adoptive T cell immunotherapy

- VACT is similar to Tumor Infiltrative Lymphocyte (TIL) but uses autologous cancer cell vaccines to condition T cells to cancer antigens
- Vaccines trigger antigen presenting cells (dendritic cells) into a state of heightened activation
- Vaccine primed mononuclear cells (MNC) are harvested via apheresis
- Infused activated T cells will stimulate an anti-cancer immune response
- Low dose IL-2 administered to further stimulate immune response



VACT: Protocol and sampling

- Four dogs undergoing VACT immunotherapy had blood sampled at selected times to assess cytokine expression in presence of target cancer cells
- Blood samples for analysis were taken prior to vaccination, two weeks after the vaccination series, and after ex vivo expansion and activation (red arrows)



Methods: cancer cell imaging and cytokine assay

Live Cell Imaging

- The IncuCyte® Live-cell Analysis System allowed for the automatic acquisition and analysis of phase contrast and fluorescence images of cells using customized software tools.
- Four images of each well using three different channels (phase contrast, green and red fluorescence) were taken every 4 h for a period of 48 h.

Sampling for Cytokine analysis

- At the end of the assay, 100 µl medium from each of the triplicate wells was transferred into one well on a separate plate to generate a pooled sample.
- From the resulting 300 µl two aliquots of 150 µl each were snap frozen in liquid nitrogen for cytokine analysis (IFNγ, TNFα, and IL-6).
- Supernatants were harvested at the end of the assay and analyzed for cytokine concentrations using the Luminex xMAP technology.

Example: T cell characteristics pre/post activation

	N=4	VIABILITY	CD4	CD8	CD25 T Cells
PRE-Activation		89%	44%	11%	< 5%
POST-Activation		81%	57%	31%	> 95%

Post Activated T cells demonstrated > 95% expression of CD25, a marker for activation

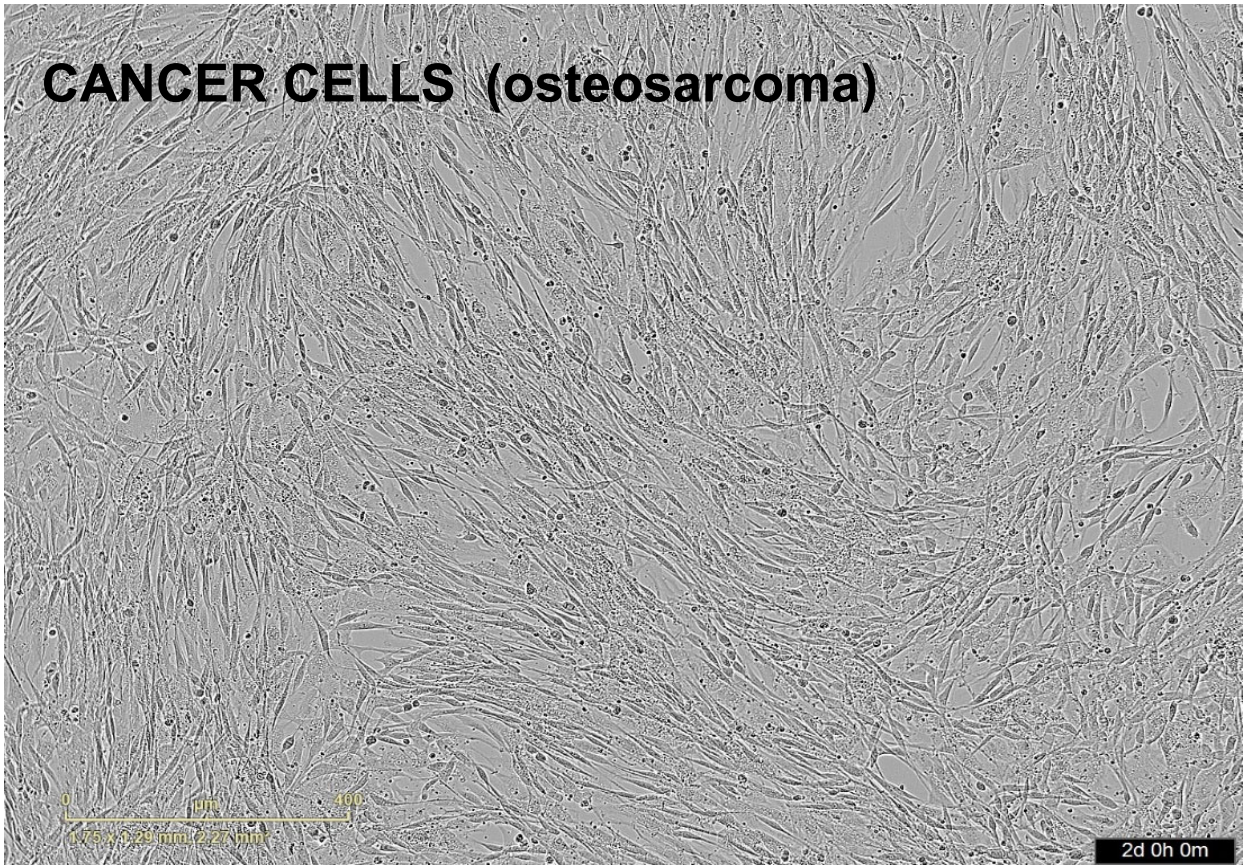
Source: Sonderegger LF, Fitzpatrick J, Wood GW, Phase I Clinical Trial to Evaluate the Safety of ELIAS Cancer Immunotherapy, Poster presented at ACVIM 2016 Conference

Results

Observations:

- Only *ex vivo* activated T cells migrated to/clustered around target cancer cells
- Confirmed activated T cell generation of desired proinflammatory and immunostimulatory cytokines (IFNγ, TNFα, and IL-6)
- Validated canine VACT process can generate cancer-antigen specific T cells
- Imaging showed that there was T cell proliferation and cancer cell death

Live Cell Imaging: normal cancer cell morphology

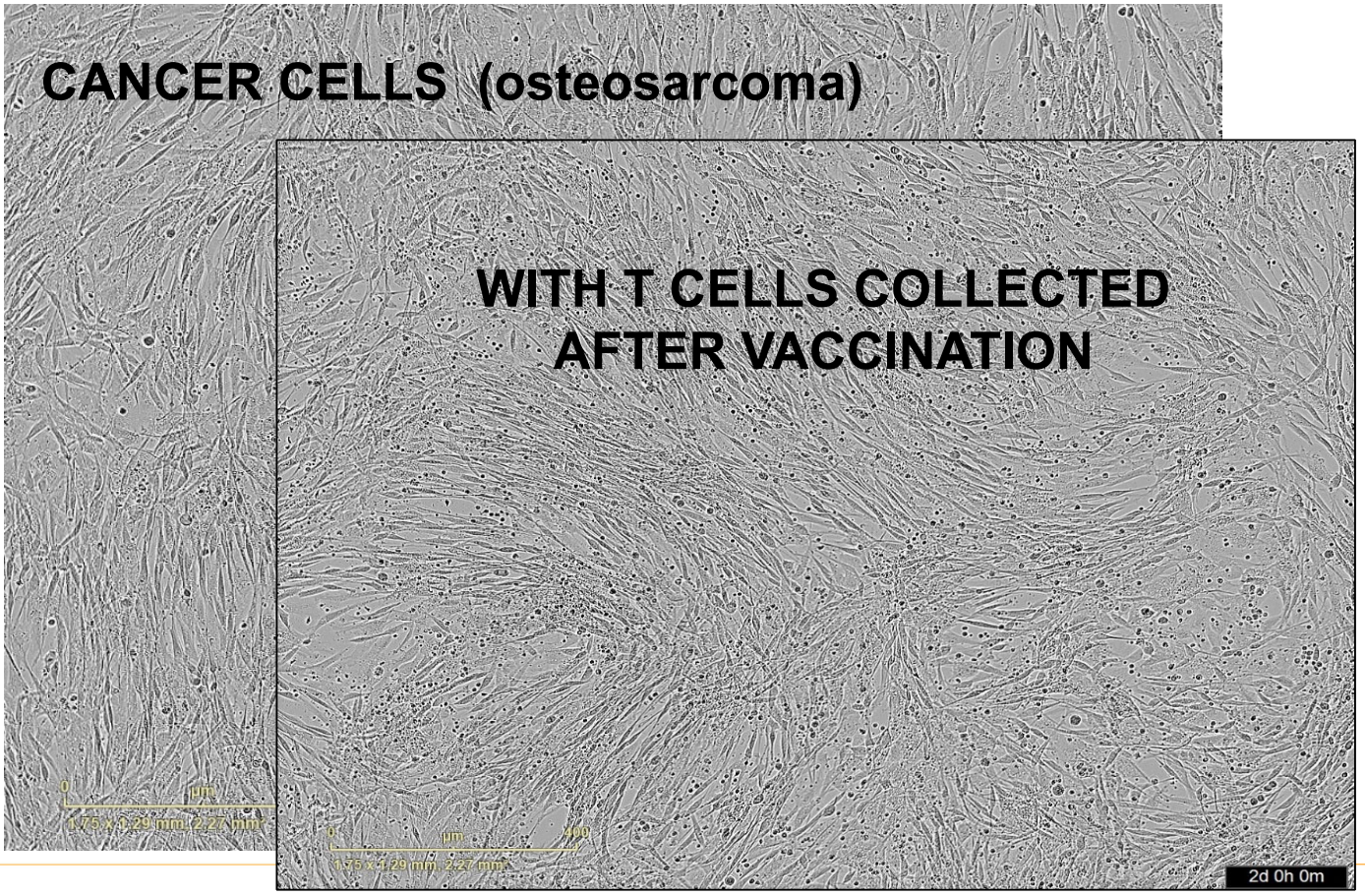


P120

Cancer cells only, seeded and allowed to grow for 48 hours.

Typical OSA spindle-like morphology seen

Live Cell Imaging: normal cancer cell morphology

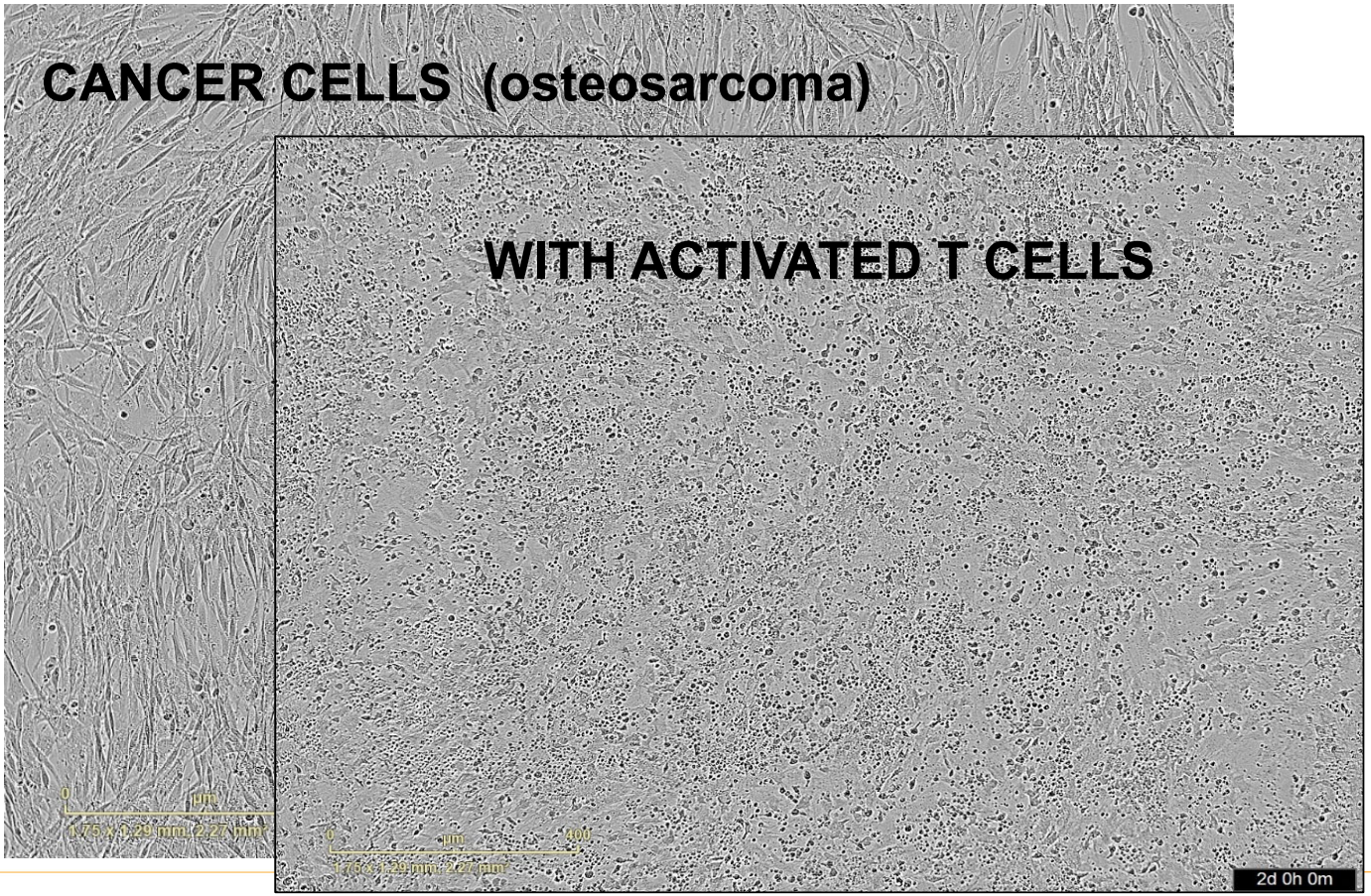


P120

Cancer cells combined with Post-vaccination T-Cells (top image) and allowed to grow for 48 hours.

Cancer cell morphology unchanged

Live Cell Imaging: normal cancer cell morphology



P120

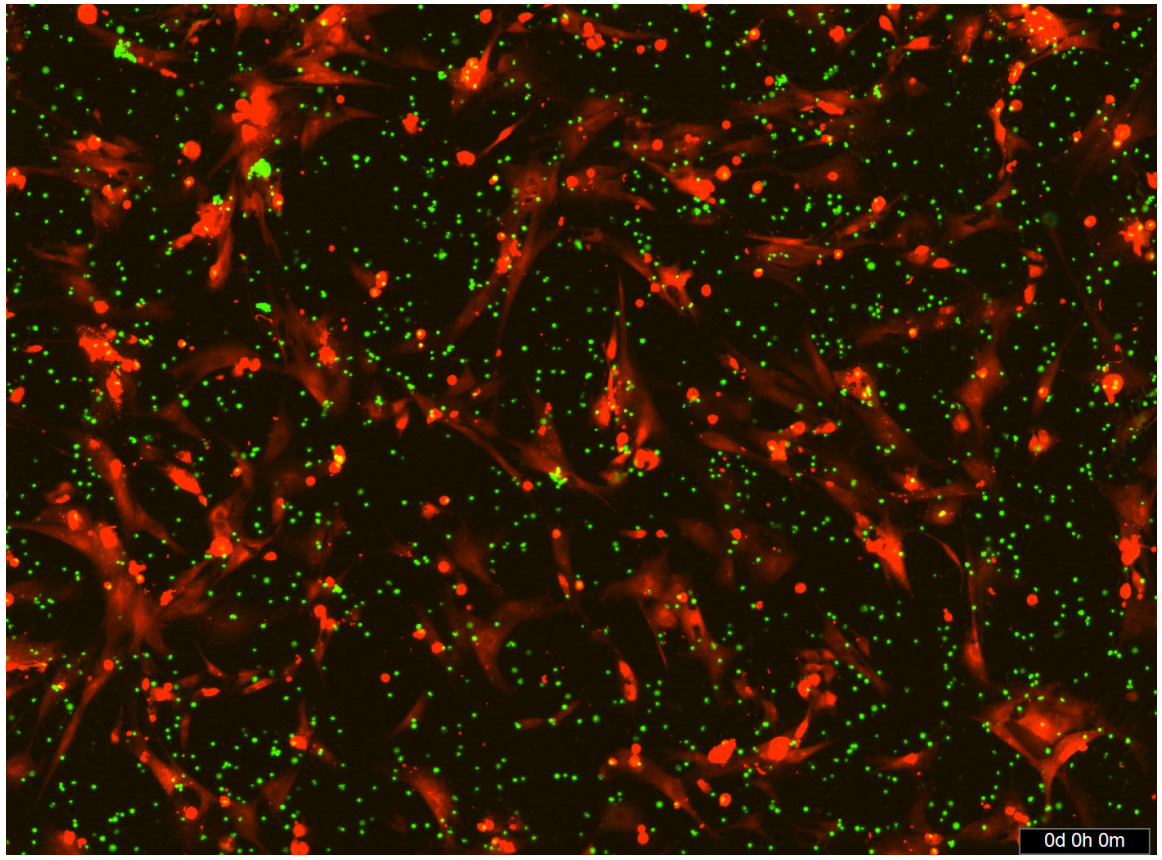
Cancer cells combined with Post-Activation T-Cells and allowed to grow for 48 hours

Post-Activation T-Cells had a disruptive effect on cancer cells

Cancer cells lose normal spindle shape

Significant proliferation of T-cells observed

Live Cell Imaging : Post Activated cells T=0



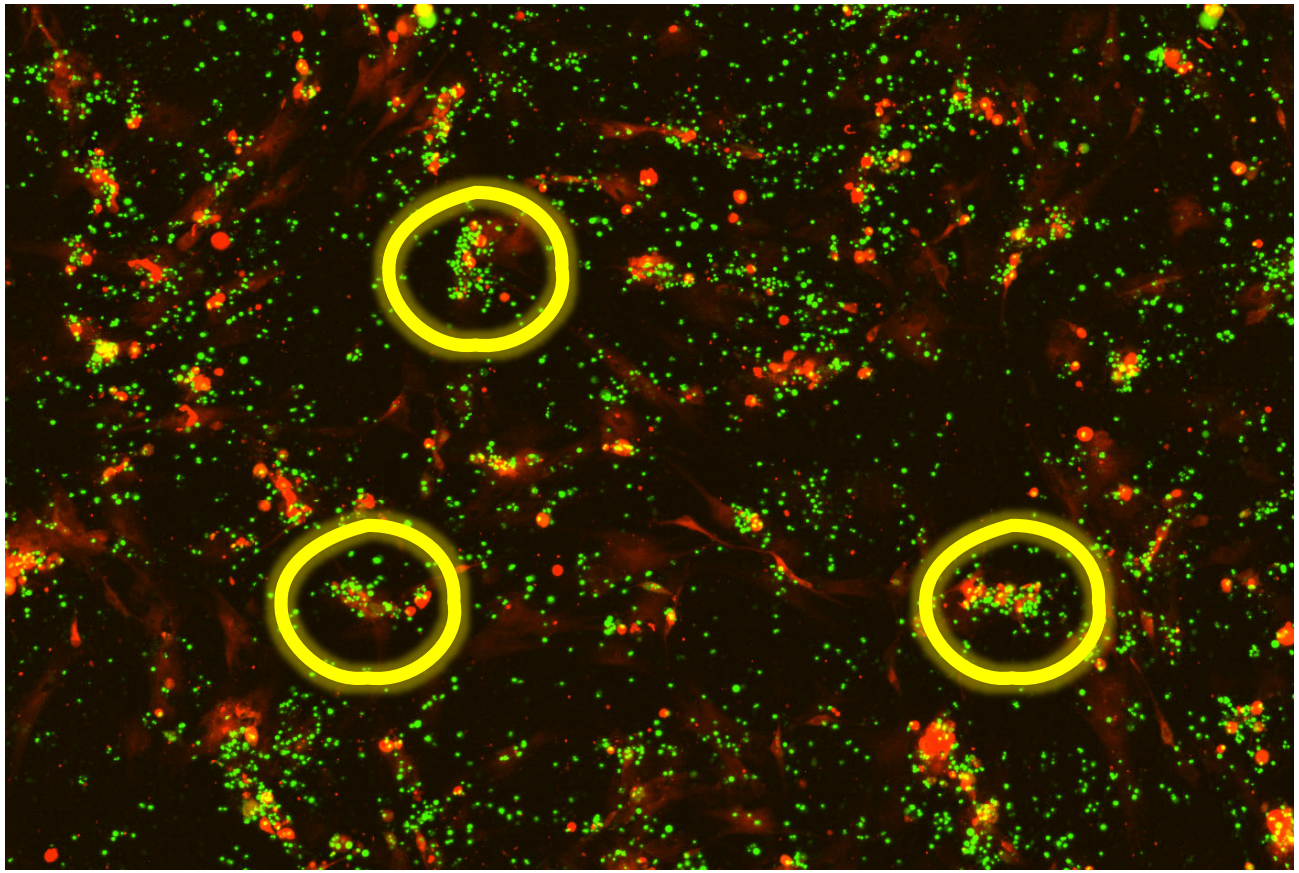
E075

Cancer cells (red) combined with Post-Activation T-Cells (green)

At d0 h0

- Widely dispersed T cells

Live Cell Imaging: Post activation T=43h



E075

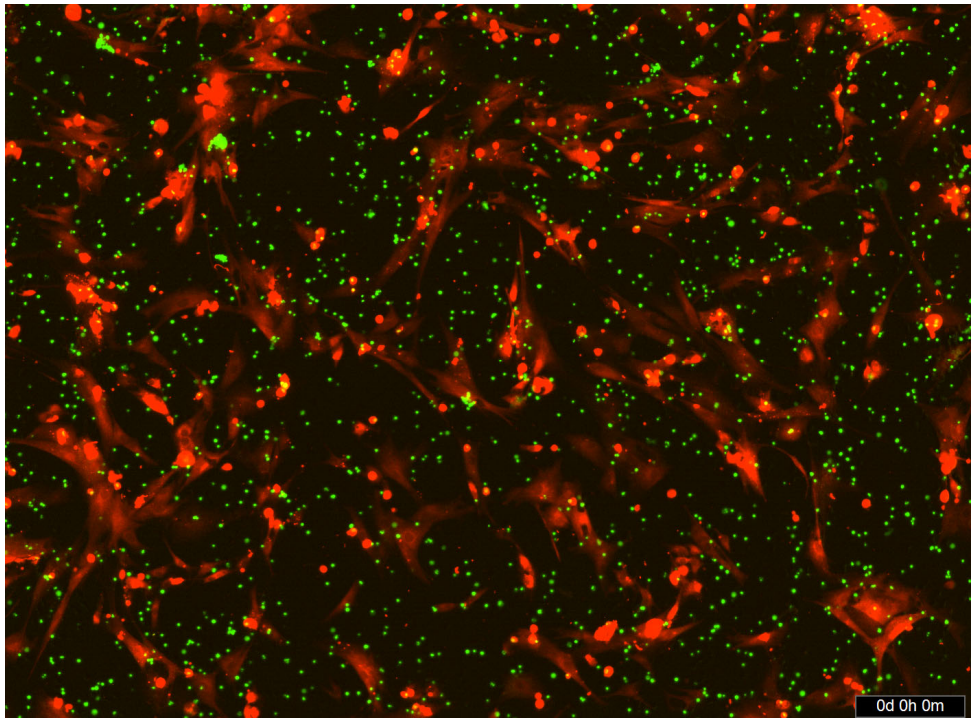
Cancer cells (red) combined with Post Activation T-Cells (green)

At d1 h19

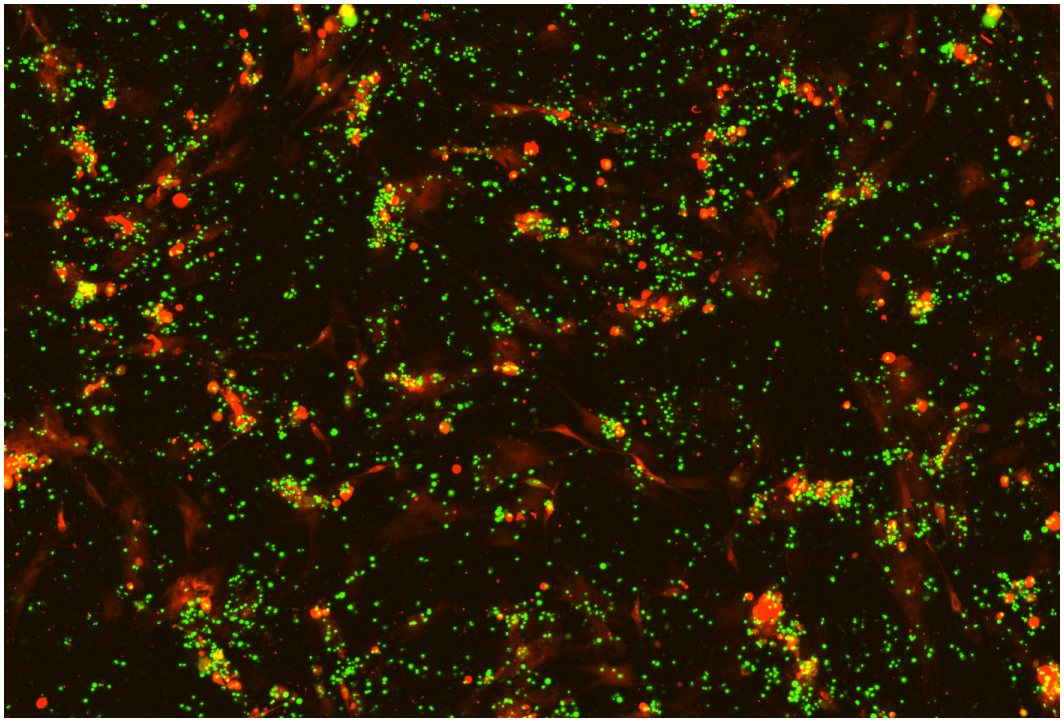
- Significant reduction in cancer cells (red)
- Significant proliferation of T cells
- T cells clustering around cancer cells (yellow circles)

Live Cell Imaging : Side-by Side Sample E075

T = 0



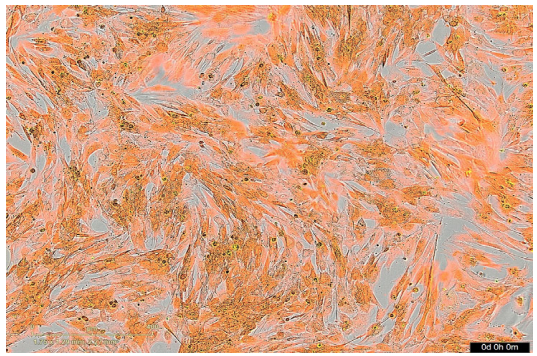
T = 43h

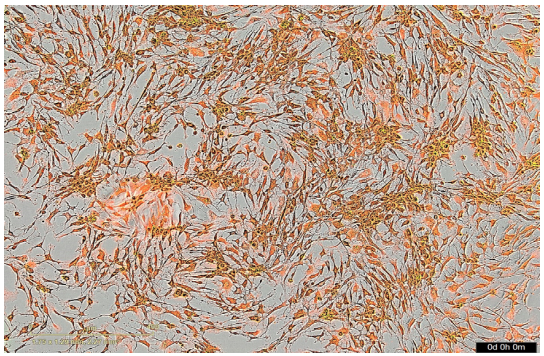


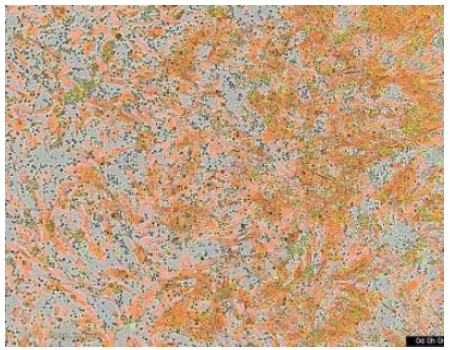
Fluorescence images of E076 at d0 and after 43h of treatment

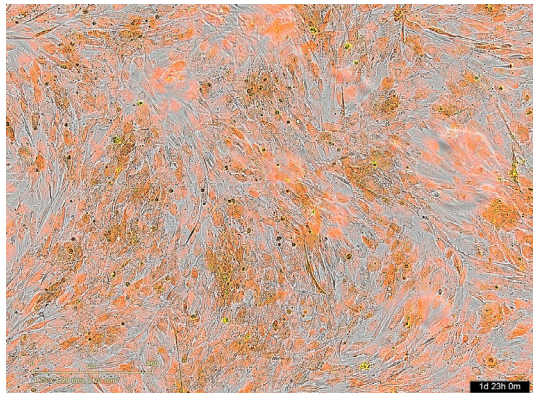
T = 0

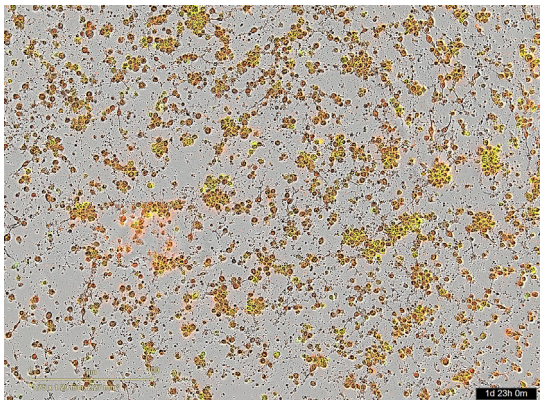
T = 43h

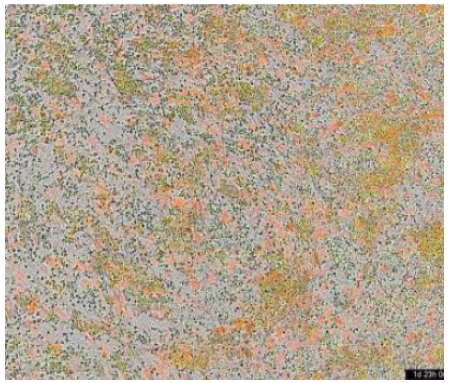










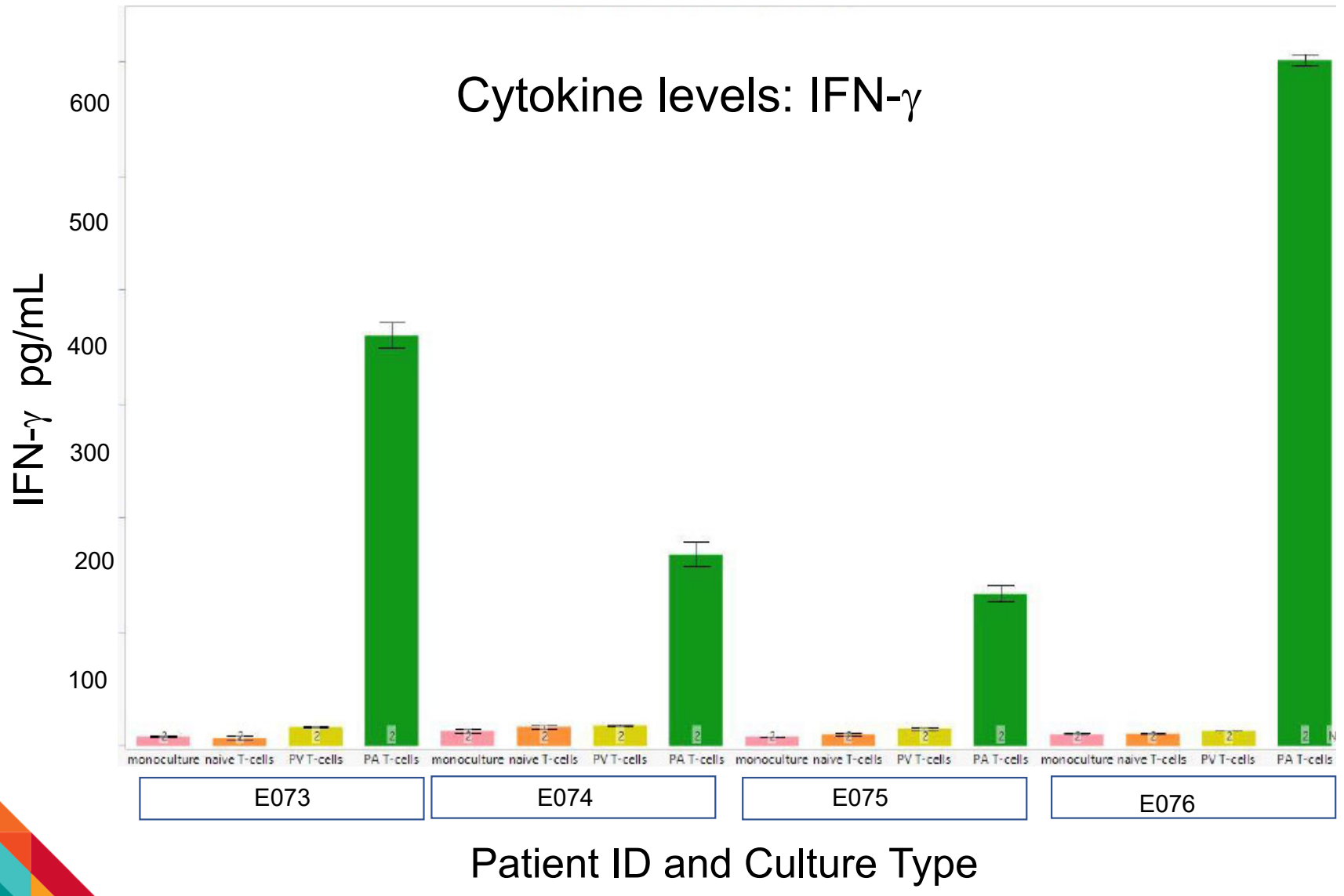


Orange = cancer cells
Green (dots) = T cells

Cancer monoculture

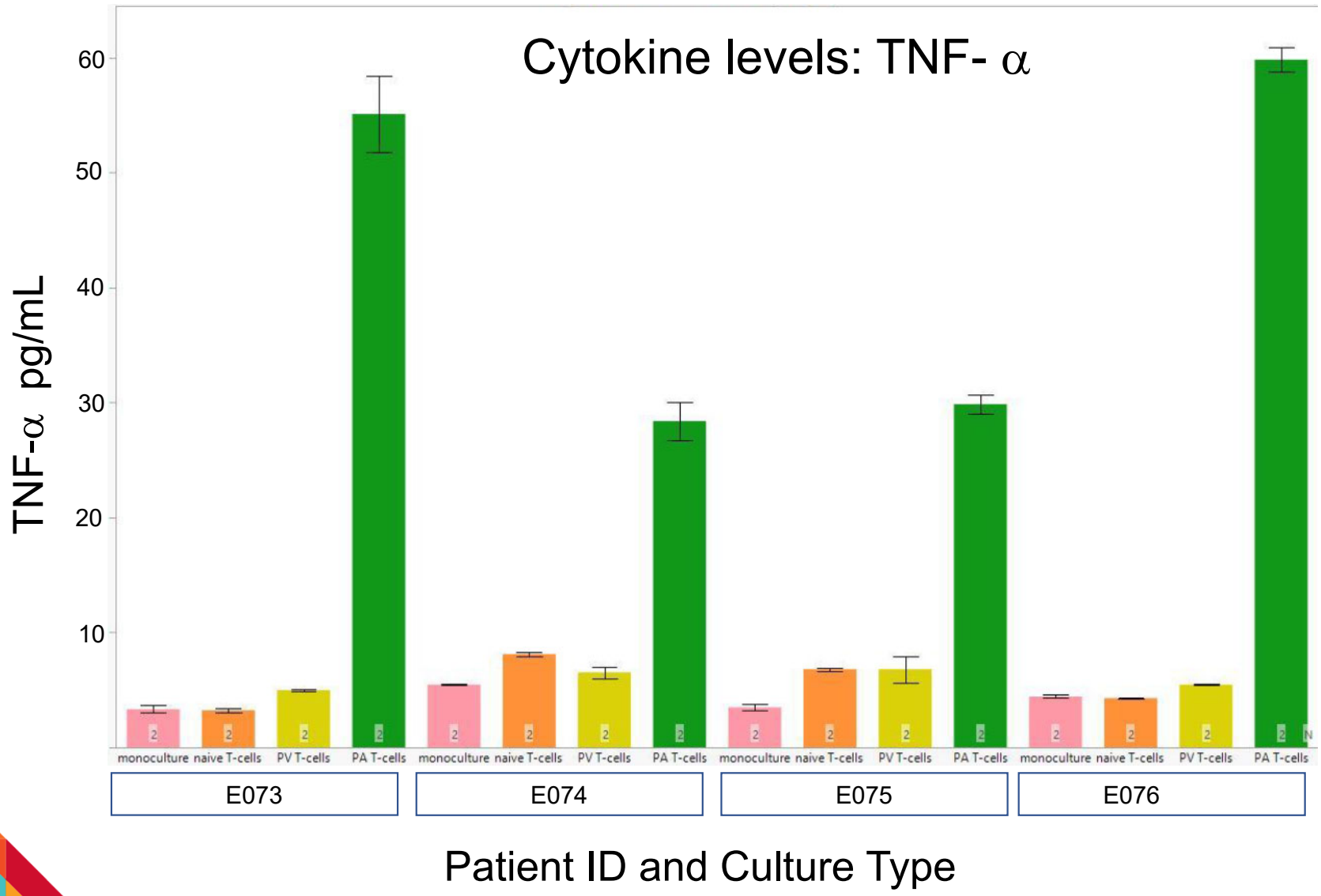
Staurosporine

Activated T cells



Minimal expression in non activated cells

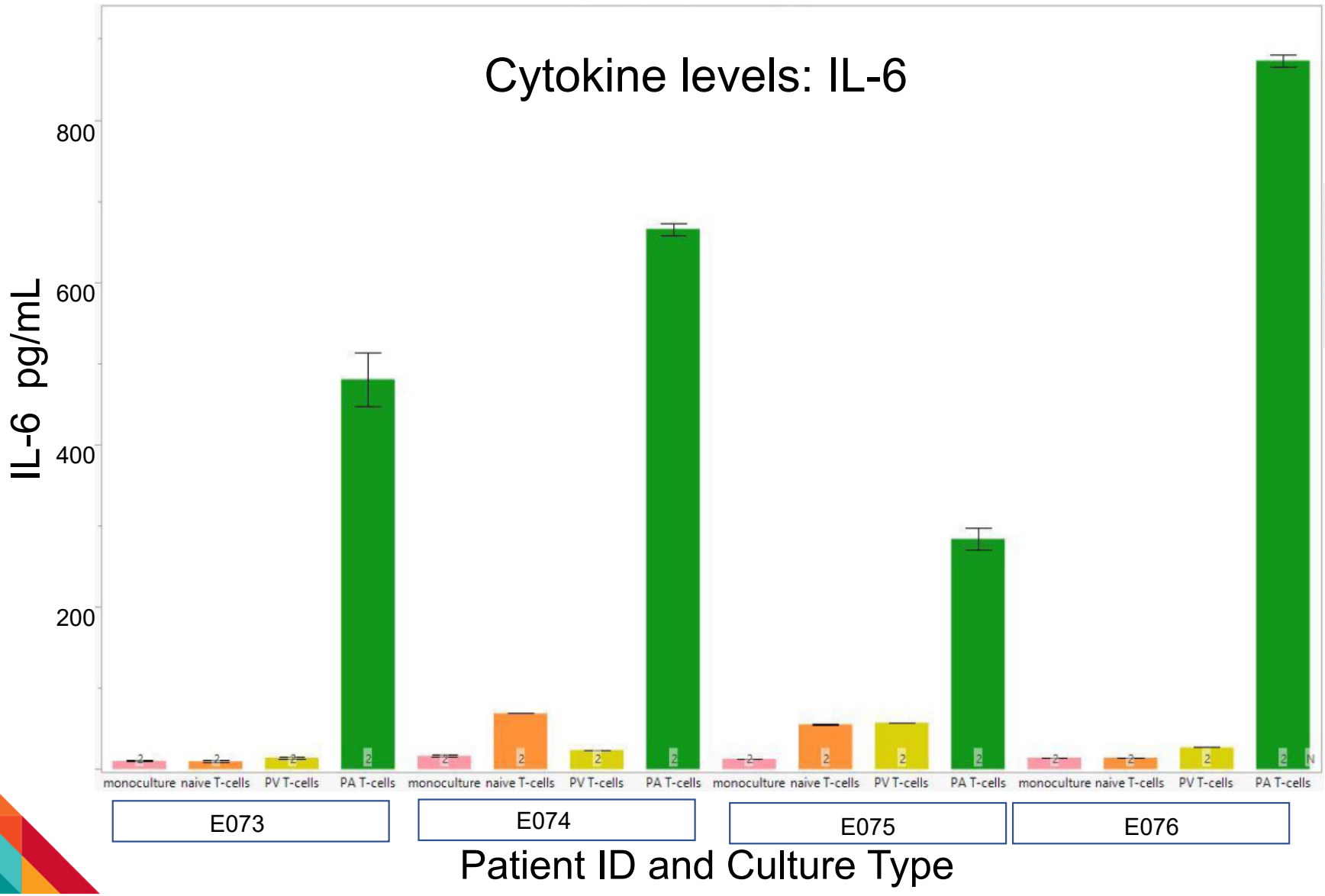
IFN-γ highest in the Activated T cells



Minimal expression in non activated cells

TNF-α highest in the Activated T cells

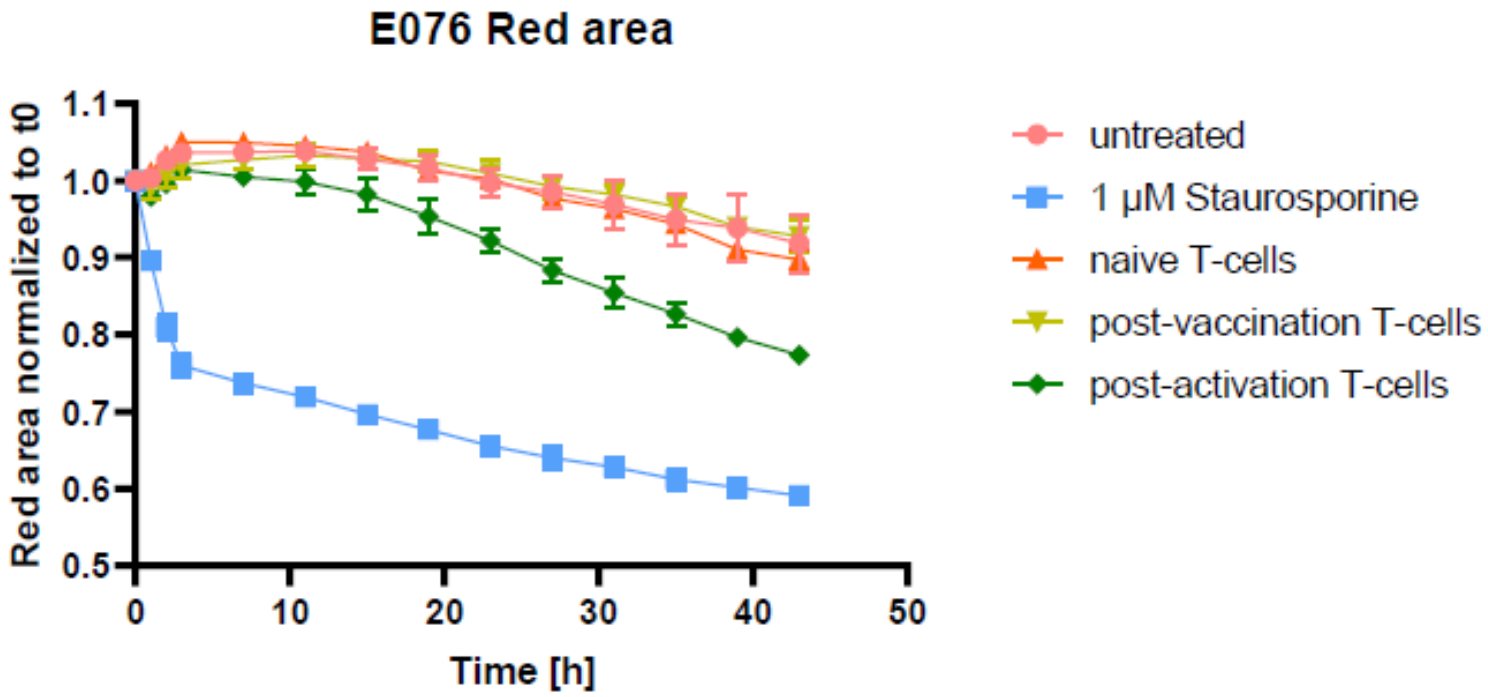
Cytokine levels: IL-6



Minimal expression in non activated cells

IFN-6 highest in the Activated T cells

Measurement of E076 Viable Cancer Cells Based on Incucyte Red Fluorescence



Only activated T cells demonstrated clear cytotoxic activity

Conclusions

- VACT immunotherapy can generate cancer-antigen specific T cells *in vivo* through the use of autologous cancer cell vaccines
- T cells conditioned by the vaccines alone will not generate an immune response unless activated
- In presence of target cancer cells, activated T cells demonstrated cytotoxicity and generated high levels of desired proinflammatory cytokines

ELIAS CANCER IMMUNOTHERAPY (ECI®) TECHNICAL SUMMARY - July 2022

The ELIAS Cancer Immunotherapy (ECI®) is built upon 50+ years of intensive scientific and medical research in rodents, canines and humans. ECI is a type of adoptive cell therapy (ACT) that is designed to stimulate the patient's immune system to recognize cancer cells as "foreign" and then mount an immune response to eliminate them. Unlike other adoptive T cell therapies (e.g., CAR-T, TIL), ECI uses attenuated autologous cancer cell vaccines to "prime" the immune cells to the cancer specific antigens. This approach allows for the harvesting of large numbers of cancer neoantigen specific immune cells. Vaccines manufactured from cancer tissue surgically collected from the patient can prime immune cells to recognize the neoantigens present on the cancer cells. Two weeks after vaccination, primed immune cells are collected from the patient via apheresis for *ex vivo* expansion. The activated T cells are reinfused into the patient and followed by a short series of low dose interleukin-2 injections to further stimulate the immune cells administered.

Efficacy demonstrated in multiple species and cancer types. In pre-clinical rodent models, the ECI approach demonstrated efficacy against a broad range of cancer types including lymphoma, melanoma, prostate, glioma, and others.[1-7] In these preclinical studies, cancers were rejected and in some cases cancer-bearing animals were permanently cured when the activated effector T cells were used to treat minimal disease.[7]

In Phase I/II human clinical trials, the ECI approach has shown efficacy in malignant glioma and renal cell carcinoma patients, with responders having their cancers put into long term remission.[8,9] In humans, this approach being developed by TVAX Biomedical is currently between Phase II/III evaluation for the treatment of glioblastoma multiforme. In 2020, TVAX Biomedical received FDA Fast Track designation for its immunotherapy based in part on key data provided from ELIAS' pilot canine study in osteosarcoma.[10,11] The positive results from ELIAS' study further supported this therapeutic approach as potentially effective in treating multiple cancer types.

Mechanism of action (MOA) has been demonstrated in an *in vitro* study using cancer cells and T cells collected from dogs being treated with ECI for osteosarcoma.[13] In the presence of their respective host cancer cells, activated T cells from vaccinated canines demonstrated cytotoxic activity and generated large levels of proinflammatory cytokines (e.g., IFN-g, TNF-a), important in stimulating an immune response against cancer. Live cell imaging (Figure 1) shows migration and clustering of activated T cells around host cancer cells.

Efficacy and safety treating canine osteosarcoma. Clinical results of a pilot study evaluating ECI in dogs (n=14) with appendicular osteosarcoma were impressive. The median survival times in this single-arm trial was 415 days, with several long-term survivors (Figure 2). These results improved upon previously reported survival results of 134 days for those treated with amputation alone and 308 days for amputation plus chemotherapy[12]. Four out of five long-term surviving dogs lived at least 2 years cancer-free, with the longest living to 5 years post-diagnosis. Another long-term survivor had a distant, cytologically confirmed osteosarcoma metastases for which no further treatment was elected. On recheck two months later, the dog was confirmed to be free of metastasis using a highly sensitive CT/PET scan.[10] This dog survived to 3 years post diagnosis and died of non-cancer causes. Further, ECI continues to demonstrate an excellent safety profile as reported in the pilot study and as seen in current patients.

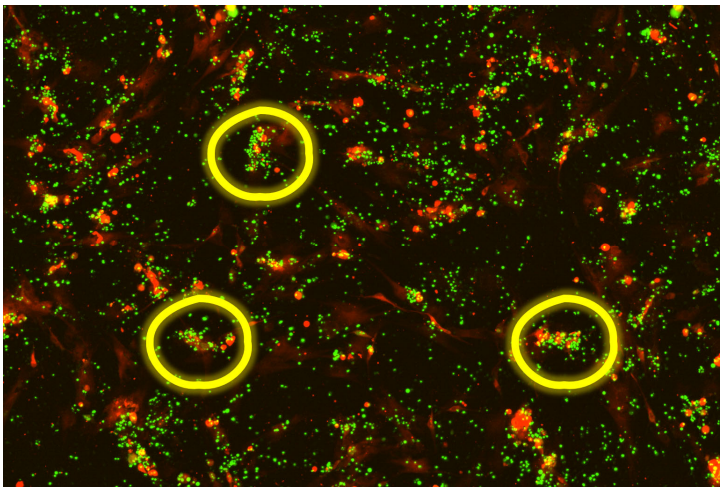


Fig 1. Activated T cells (green) migrating to and clustering around host cancer cells (red). Antigen-specific T cells generate immunostimulatory cytokines and demonstrate cytotoxic activity toward the cancer cells.

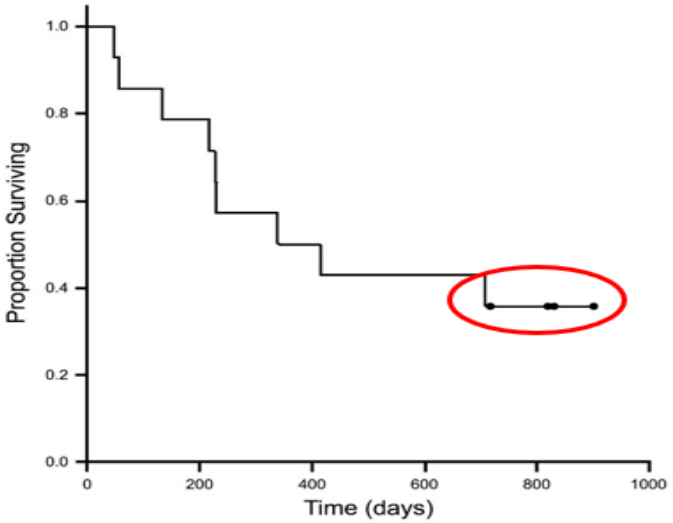


Fig 2. Kaplan Meier curve showing overall survival time for all dogs on an intent-to-treat basis undergoing ECI therapy for osteosarcoma. Overall median survival time (n=14 dogs) was 415 days. Red circle highlights long-term surviving dogs at study conclusion (> 700 days).

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ELIAS ANIMAL HEALTH