

# Sequential tracking of PD-L1 expression and RAD50 induction in CTCs and circulating stromal cells of lung cancer patients during treatment with radiotherapy

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## ABSTRACT

It is hypothesized that PD-L1 expression can be induced by radiotherapy (RT) and may be a mechanism for resistance to RT, and immunotherapy. However, repetitive biopsies for monitoring PD-L1 expression in tumor and/or stroma is not feasible. Sequentially assessing PD-L1 expression on multiple cancer associated circulating cells during treatment may be a way to assess the efficacy of RT and immunotherapy. RAD50 is a DNA repair gene that can be used to track tumor response to radiation. We therefore evaluated PD-L1 expression and RAD50 induction in CTCs, CTCs undergoing Epithelial to Mesenchymal Transition (EMTCTCs), and Cancer Associated Macrophage-Like Cells (CAMLs) in lung cancer patients (pts) before and during RT to track expression changes of these markers.

## INTRODUCTION

The presence of circulating tumor cells (CTCs) is an indicator of metastatic disease, used to monitor therapy response and predict outcomes in late stage patients.<sup>1-4</sup> However, CTCs are not equally found in all tumor types, and the low frequency makes the tracking of therapeutic response difficult.

Cancer Associated Macrophage-like cells (CAMLs) are a newly-defined circulating immune cell type, described as a subtype of circulating stromal cells. CAMLs express actionable drug targets (e.g. TIE-2, CXCR4, PD-L1, etc.), have been shown to be present in all stages of cancer, are responsive to cancer treatments, and are found in multiple cancer types.

EMT-like cells are found in almost all patients with solid malignancies and are theorized to be the aggressive CTC subtype responsible for tumor metastases. Typically, EMT-like cells are identified by their down regulation of EpCAM, and/or Cytokeratin, and up regulation of Vimentin, and/or N-Cadherin.<sup>1-4</sup>

We previously reported that CellSieve™ microfilters rapidly and efficiently isolate the three most common circulating cancer associated cells (CAMLs, CTCs, and EMTs) from peripheral blood, making it possible to study all cell types in conjunction with, and in relation to, therapy response in a variety of malignant diseases.<sup>3-4</sup>

Figure 1. CTCs, CAMLs, and EMTCTCs can all be isolated from a single tube of blood

(a) Typical CTCs are not commonly found in NSCLC patients

(b) EMTCTCs are more common in NSCLC patients

(c) CAMLs are common and increase in patients undergoing treatment. Scale=10um

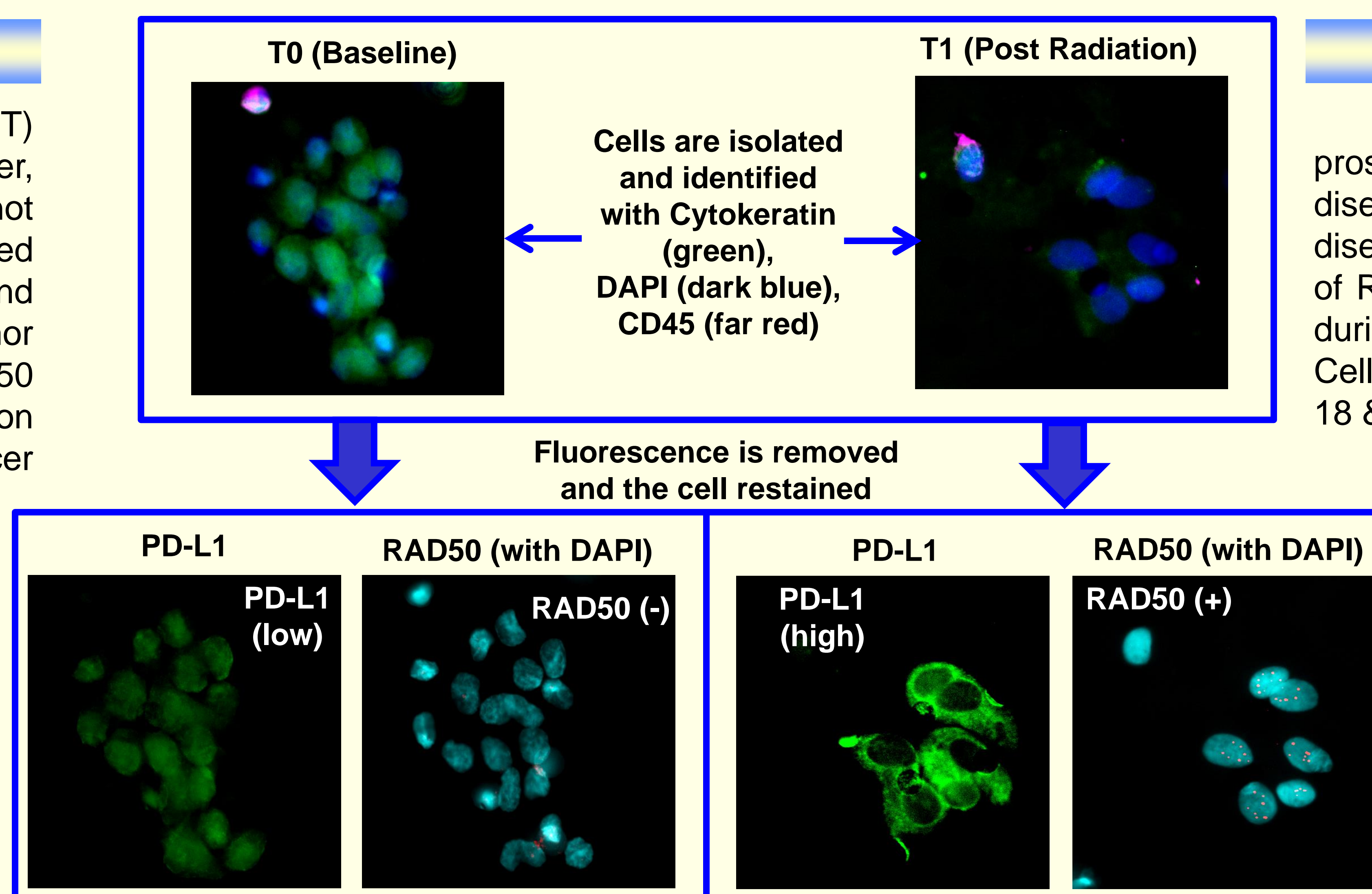
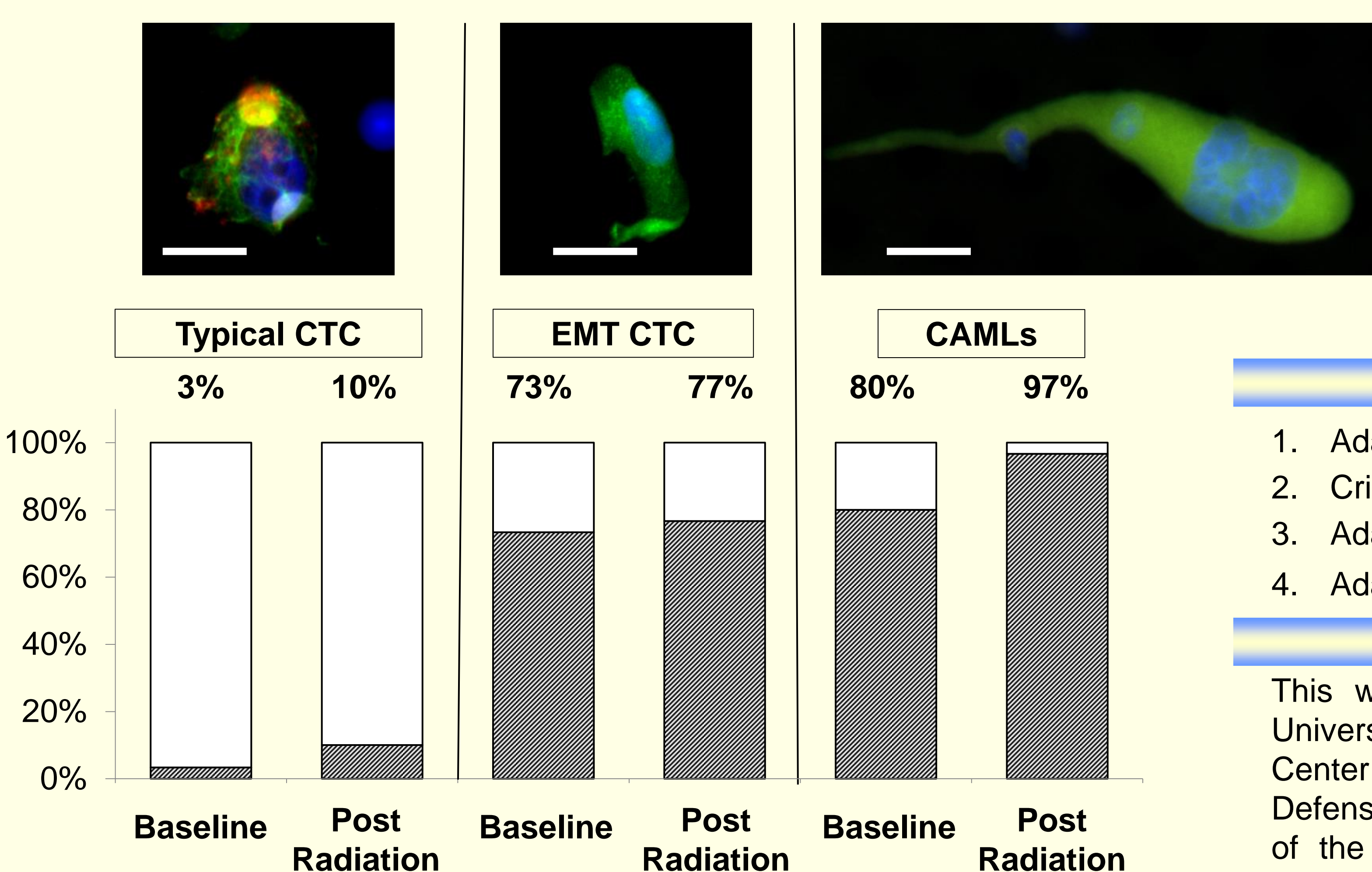


Figure 2. Staining for PD-L1 (green) and RAD50 (red) showing high and low expressions. RAD50 foci are seen as fine red dots on a light blue nuclei.

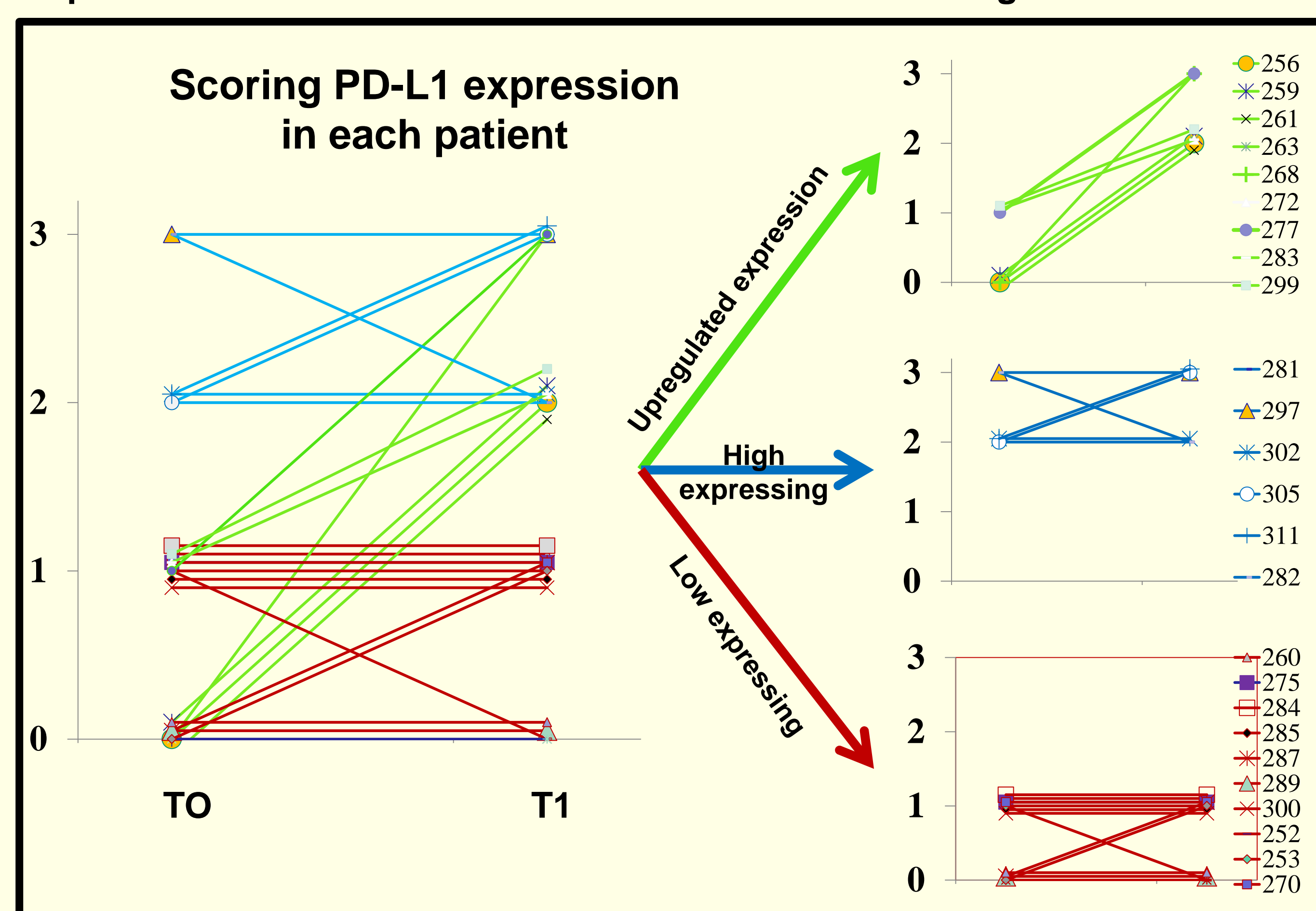


Figure 3. PD-L1 expression before (T0) and after (T1) chemoradiation for 30 Stage I-IV NSCLC patients.

## References

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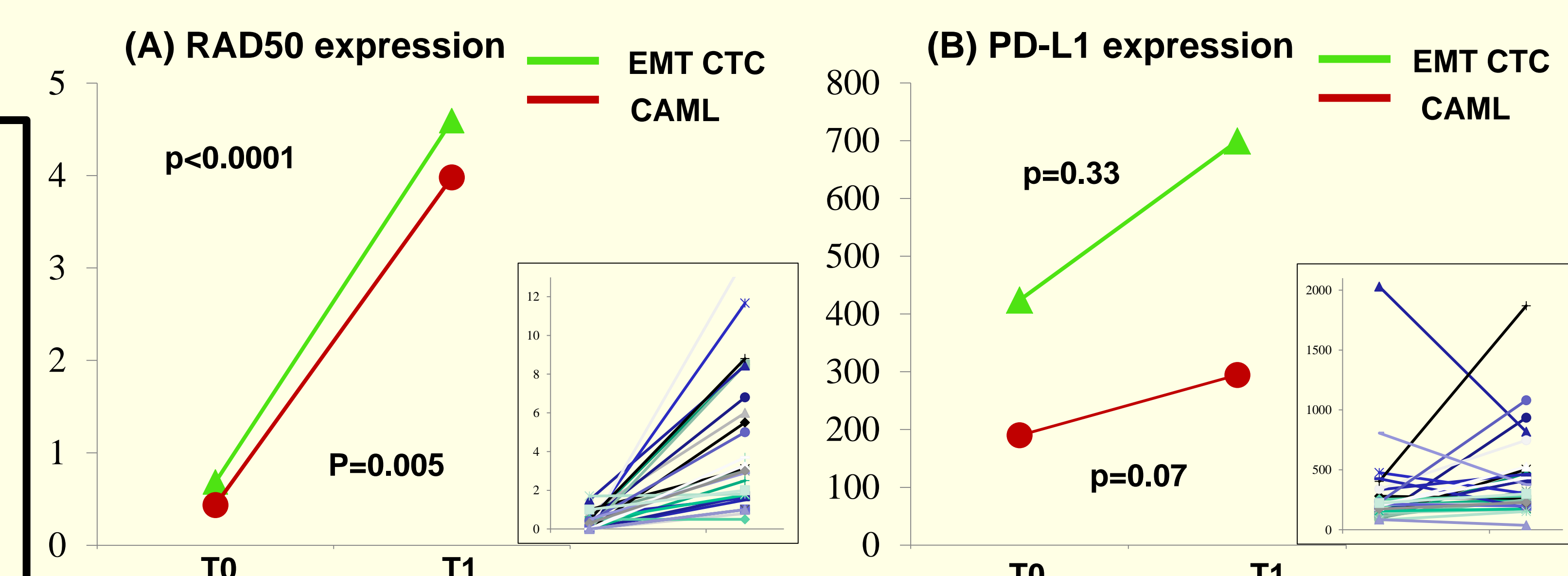
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## MATERIALS & METHODS

Thirty pts with stage I-IV lung cancer were included in this prospective pilot study. Four pts received radiation therapy for stage I disease and 26 other pts received chemoradiation for stage II-IV disease. Baseline blood samples (7.5 ml) were drawn prior to the start of RT (T0), and a second blood sample was drawn at a follow up visit during RT; for a total of 60 samples. Blood was processed using CellSieve™ microfiltration (Creatv MicroTech); stained for cytokeratin 8, 18 & 19 and CD45; and imaged.

Using the QUAS-R (Quench, Underivatize, Amine-Strip and Restain) technique to remove fluorescence signal, all cells were restained for RAD50-AlexaFluor550 and PD-L1-AlexaFluor 488, along with DAPI nuclear stain. The RAD50 foci numbers within nuclear regions were quantified. PD-L1 pixel intensity was measured and grouped into 4 IHC groups: 0-negative (pixel average 0-185), 1-low (pixel average 186-299), 2-medium (pixel average 300-750), and 3-high (pixel average 751+).

Figure 4. (A) RAD50 expression and (B) PD-L1 expression pre (T0) and post (T1) radiotherapy.



## RESULTS

- One or more cytokeratin positive cell (i.e. CTC, EMTCTC, or CAML) was found in all but 2 samples (97%).
- EMTCTCs and CAMLs were commonly found in patient samples.
- 9 pts had low/no PD-L1 at T0, but a 2/3+ at T1.
- 15 pts had low/no PD-L1 at T0, remaining low at T1.
- 6 pts had high PD-L1 at T0, remaining high at T1.
- During radiotherapy, RAD50 foci increased from 0.6 at T0 to 4.3 at T1.

## CONCLUSIONS

- Microfiltration captures CAMLs, EMTCTCs and CTCs, irrespective of surface marker expression.
- PD-L1 expression and RAD 50 foci are quantifiable and trackable in both EMTCTCs and circulating stromal cells.
- EMTCTCs and CAMLs had a correlative response to radiotherapy.
- Sequential tracking of CTCs and immune-related cells from the primary lung tumor is feasible using blood based biopsies.
- There was no correlation between RAD50 induction and PD-L1 expression.