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Changes in the type of circulating cancer associated macrophage-like cells during and after radiation therapy is associated with progression in thoracic cancers

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ABSTRACT

Personalizing therapy in cancer patients requires individualized determination of the likelihood of cancer progression both before and during therapy. It has been suggested that a recently described circulating cancer associated stromal cell type called Cancer Associated Macrophage-Like cells (CAMLs) might be used predict patient survival before and after treatment. To test this hypothesis, we assessed the predictive value of monitoring changes in CAMLs as it relates to disease progression, or survival, during and after definitive radiotherapy in unresectable non-small cell lung cancer (NSCLC) and esophageal cancer (EC).

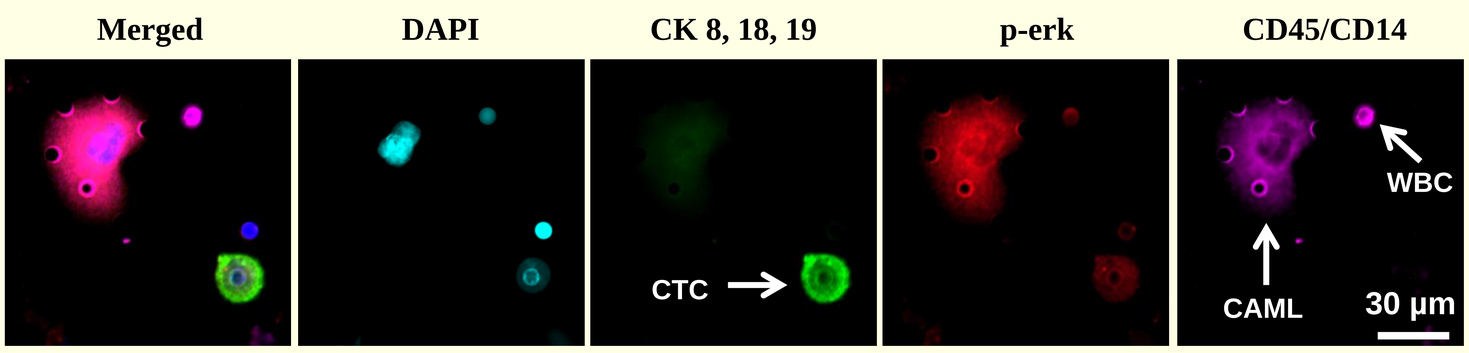

Figure 1. Example of CTC isolated with a CAML in a breast cancer patient. CTCs are Cytokeratin positive (green) and CD45/CD14 negative. In contrast, CAMLs are CD45/CD14 positive (purple) and may be weakly positive for Cytokeratin (green). White blood cells (WBCs) are normal sized CD45/CD14 positive cells.

INTRODUCTION

CAMLs are specialized myeloid cells transiting the circulation of patients in all stages of cancer. They are responsive to cancer treatment and are found in multiple cancer types[1,2]. However, though seen by numerous groups, these cells have remained largely unstudied, and their clinical and biological value in malignancies remains uninvestigated. Size exclusion is a technique for isolating large cells from peripheral patient blood irrespective of their surface marker expression. CellSieve™ microfilters are size exclusion membranes capable of rapidly and efficiently isolating CAMLs from whole blood, making it possible to study CAML subtypes in conjunction with and in relation to malignant disease[1-4].

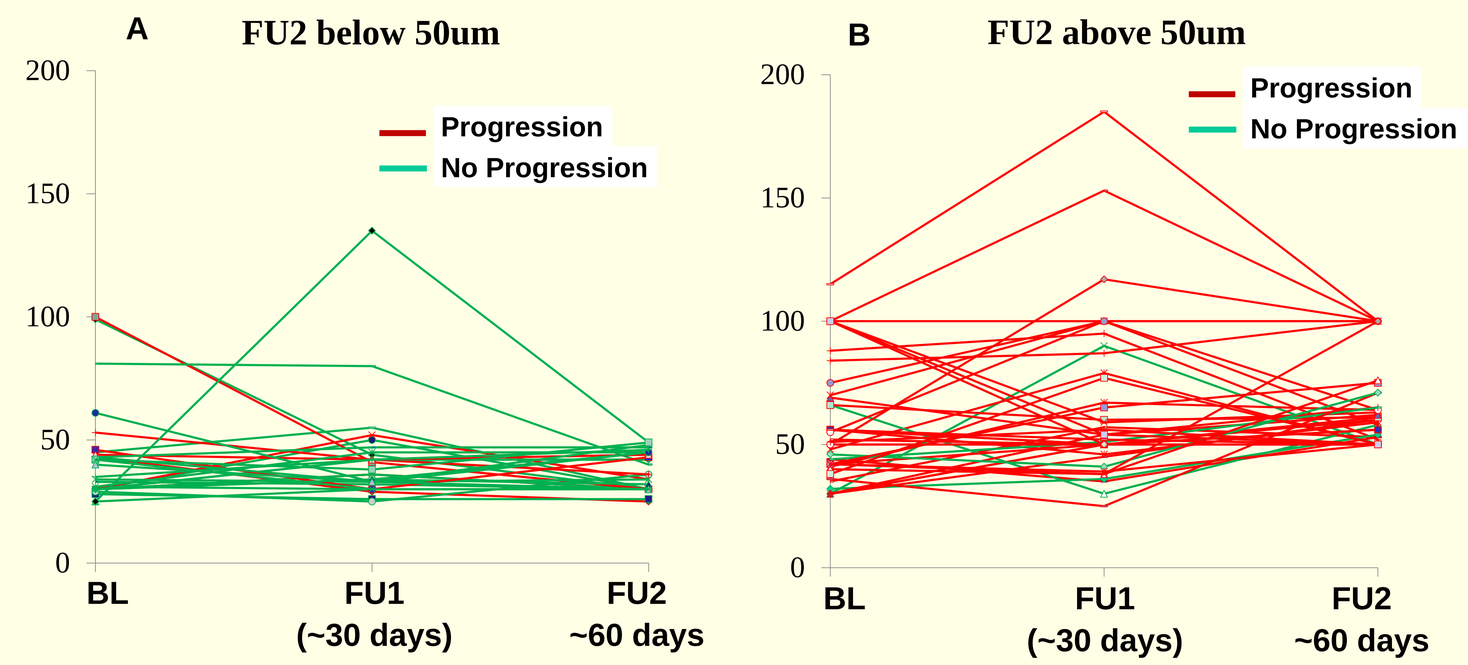

Figure 2. Largest CAML found in samples at each of 3 time points; BL, FU1 and FU2; assessed by CAML size at FU2. A. Only 21% of patients with <50 µm at FU2 progressed within 24 months. B. 89% of patients with ≥50 µm at FU2 progressed within 24 months.

References

1. Adams DL, et al "Circulating giant macrophages as a potential biomarker of solid tumors." Proc Natl Acad Sci, 111(9):3514-3519. 2014
2. Cristofanilli M, "Liquid Biopsies in Solid Tumors" *Springer Intl Publish.* 2017
3. Adams DL, et al. "Sequential tracking of PD-L1 expression and RAD50 induction in circulating tumor and stromal cells of lung cancer patients undergoing radiotherapy" Clin Can Res, 23(19): 5948-5958. 2017

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MATERIALS & METHODS

A 2 year prospective single blinded pilot study was run to assess the role of CAMLs in predicting progression free survival (PFS) in patients after completion definitive radiotherapy, or chemoradiotherapy, in unresectable NSCLCs or ECs. Based on prior data which demonstrated the prognostic role of baseline CAMLs ≥50 µm, we hypothesized that changes in CAMLs size during therapy might be predictive of disease progression. We analyzed 52 NSCLC (stage I, n=7, stage II, n=7, stage III, n=29, and stage IV, n=9) and 21 esophageal cancer (stage III n=20 and Stage IV (n=1) patients. Baseline (BL) blood sample was obtained prior to start of radiation, a 2nd blood sample (T1, ~30 days) was taken midway during therapy and a 3rd sample (T2, ~60 days) was taken at the end of radiotherapy. Whole 7.5 mL peripheral blood was processed using CellSieve™ microfiltration and CAML sizes were quantified. CAMLs <49 or ≥50 µm sizes were quantified but the observer was blinded to the disease type or clinical information. The CAML measurement was then used to evaluate PFS hazard ratios (HRs) by censored univariate & multivariate analysis at each time point.

RESULTS

- CAMLs were in 97% of BL samples (2.9 CAMLs/7.5 mL of blood)
- At BL, CAMLs ≥50 µm had reduced PFS
  - NSCLC (HR=2.9, 95%CI 1.3-6.2, p=0.015)
  - EC (HR=3.0, 95%CI 0.9-9.9, p=0.14).
- At T1, CAML size ≥50 µm had a reduced PFS
  - NSCLC (HR=5.0, 95%CI 2.3-10.9, p<0.001)
  - EC (HR=4.0, 95%CI 1.2-13.2, p=0.05)
- At T2 patients CAML size ≥50 µm had further reduced PFS
  - NSCLC (HR=7.1, 95%CI 3.4-14.8, p<0.001)
  - EC (HR=5.6, 95%CI 1.6-18.8, p=0.01).
- In a multivariable analysis CAMLs were the most significant independent prognostic variable.
- ≥50 µm CAMLs at BL was 70% accurate at predicting progression within 24 months
- ≥50 µm CAMLs at T2 was 84% accurate at predicting progression within 24 months

CONCLUSIONS

- Giant CAMLs were prognostic both at pretreatment baseline as well as any enlargement that happens during and after therapy in NSCLC and EC
- Giant CAMLs could represent a population of tumor stroma cells that promote tumor progression
- Monitoring the presence of giant CAMLs through the course of RT could be predictive of cancer progression or death
- Prospective validation of giant CAMLs as a blood-based biomarker for risk stratification is pending though a R43/SBIR

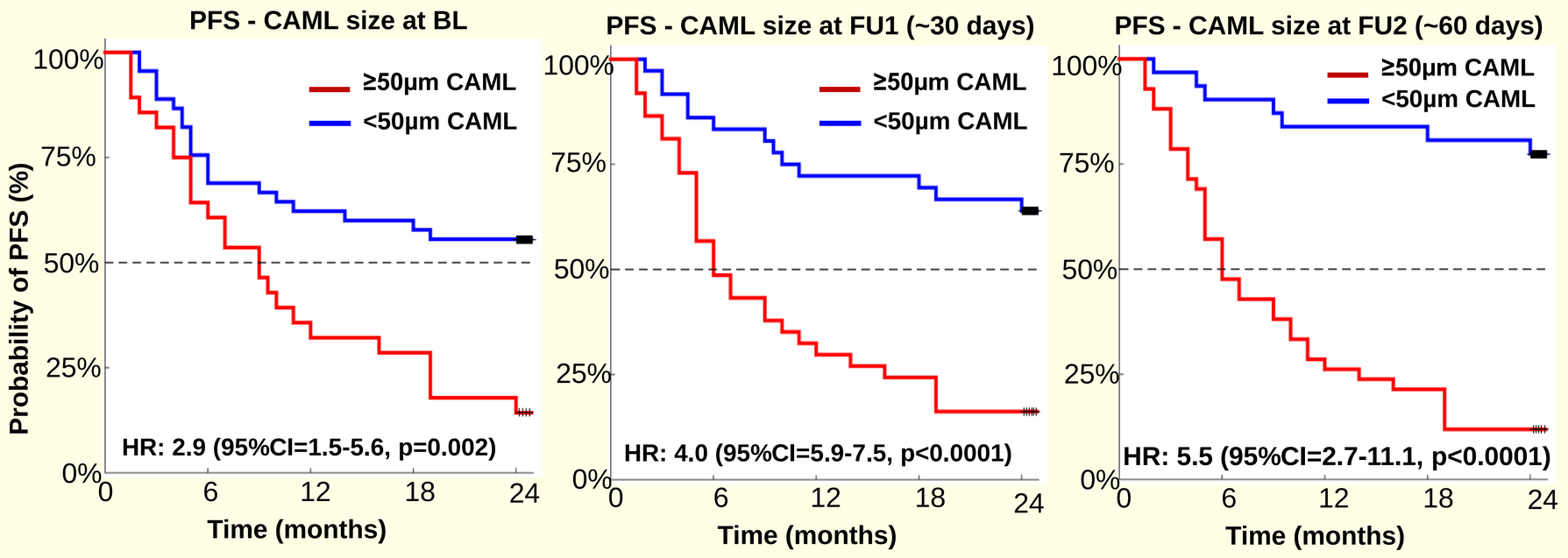

Figure 3. Kaplan-Meier plots comparing <50 µm and ≥50 µm at each of 3 time points (BL, FU1 and FU2)