

Identification of circulating immune factors as predictive biomarkers of CDK4/6i treatment efficacy in advanced breast cancer (InMaM)



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Background

CDK4/6 inhibitors (CDK4/6i) have revolutionized the treatment of advanced breast cancer (ABC) patients. Despite significant improvements in survival, pointing towards antitumor immune response, reliable biomarkers of response are needed. Here, we identified a group of circulating blood immune factors that determine CDK4/6i efficacy and may be useful as predictive biomarkers.

Methods

Luminex® technology was used to evaluate levels of soluble circulating factors in peripheral blood from 68 ER+/HER2 ABC patients treated with CDK4/6i at different time-points: prior to treatment initiation (Baseline), after 4-6 months under treatment (INT) and at progression or last follow-up (PROG/LFUP). Response was classified in good (GR) or poor (PR) based on hormone sensitivity and progression-free survival (PFS) according to data from pivotal studies (Figure 1). T-test comparisons were used to identify factors associated to each response group and Kaplan Meier and Cox regression models to assess association with survival.

Patient Characteristics	n (%)
Previous Lines of Therapy	
1 Line (1L)	39 (57.4%)
2 Line (2L)	9 (13.2%)
> 2L	20 (29.4%)
M1 Recurrence Status	
1L De novo	7 (10.3%)
1L >12m from adjuvant therapy	20 (29.4%)
1L <12m from adjuvant therapy and >1L previous hormonal therapy for M1	24 (35.3%)
Chemotherapy (Ch)	17 (25.0%)
Localization of M1	
Visceral	31 (45.6%)
Hepatic	18 (26.5%)
Only bone	19 (27.9%)
Survival endpoints, months (m)	
Median PFS	12.9 m
Median OS	42.8 m

Table 1. Clinical characteristics of the patients included in the study. n, number of patients; L, number of lines of therapy; m, months; M1, metastatic.

Results

To streamline the consideration of patient-specific characteristics, we categorized patients into either good or bad efficacy groups based on progression-free survival data from pivotal trials, taking into account treatment regimen, therapy line, hormonal sensitivity, or administration of prior chemotherapy.

GOOD	BAD
PFS > 24 months Without prior treatment for metastatic disease, de novo onset, relapse >12 months from adjuvant endocrine treatment. Based on MONARCH3, MONALEESA2/7 and PALOMA2	PFS < 24 months
PFS > 12 months Progression during HT for advanced disease, progression during adjuvant endocrine treatment. Based on MONARCH2, MONALEESA3 and PALOMA3	PFS < 12 months
PFS > 7 months Previous chemotherapeutic treatment. Based on MONARCH1 and PALOMA3	PFS < 7 months

Figure 1. Efficacy classification of the RESIST cohort recruited in ICO-Badalona (n=100). The 100 patients in the cohort were reclassified according to distinct clinical criteria from the pivotal clinical trials of CDK4/6i. 91 patients had sufficient follow-up at the time of the last analysis. The cut-off point to determine good or poor efficacy varied depending on the patient's treatment status. Patients were divided in three distinct groups: hormone-sensitive if de novo metastatic onset and no prior oncologic treatments or with relapses beyond 12 months after hormonal treatment; hormone-resistant if they relapsed during adjuvant hormonal treatment, within the first 12 months post-adjuvant treatment, or after first-line hormonal treatment as monotherapy or patients who had received chemotherapy for metastatic disease prior to CDK4/6i. For each group, the duration cut-off to be considered good or poor efficacy was determined based on studies with similar clinical characteristics (represented in the figure).

Clinical characteristics based on efficacy of CDK4/6i treatment

Patients were classified in two groups of different efficacy to CDK4/6i treatment, regardless of the line of treatment, metastatic disease and previous treatments (situations that could lead to a bias in the treatment efficacy).

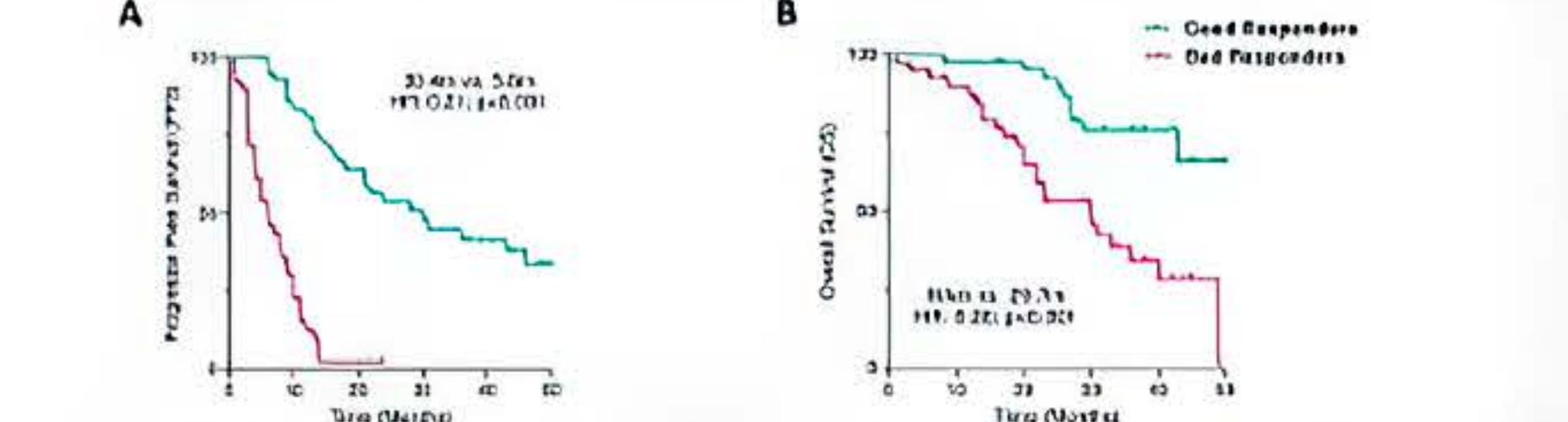


Figure 2. Survival analysis depending on clinical efficacy groups. (A) Progression free survival (PFS) and (B) overall survival (OS) based on the CDK4/6i treatment clinical efficacy. p-value calculated using log-rank test.

Identification of circulating immune factors related to CDK4/6i efficacy in ABC patients' plasma samples prior to treatment

Twenty-nine distinct circulating blood factors, including pro- and anti-inflammatory cytokines were assessed in basal samples of a subcohort (N=20, 10 Good Responders and 10 Poor Responders). Supervised hierarchical clustering identified 3 distinct clusters of cytokines differentially expressed depending on treatment efficacy. The most informative cytokines were selected for further testing in the extended cohort (N=68), together with soluble immune checkpoints (PD-1, CTLA-4, TIM3 and LAG3).

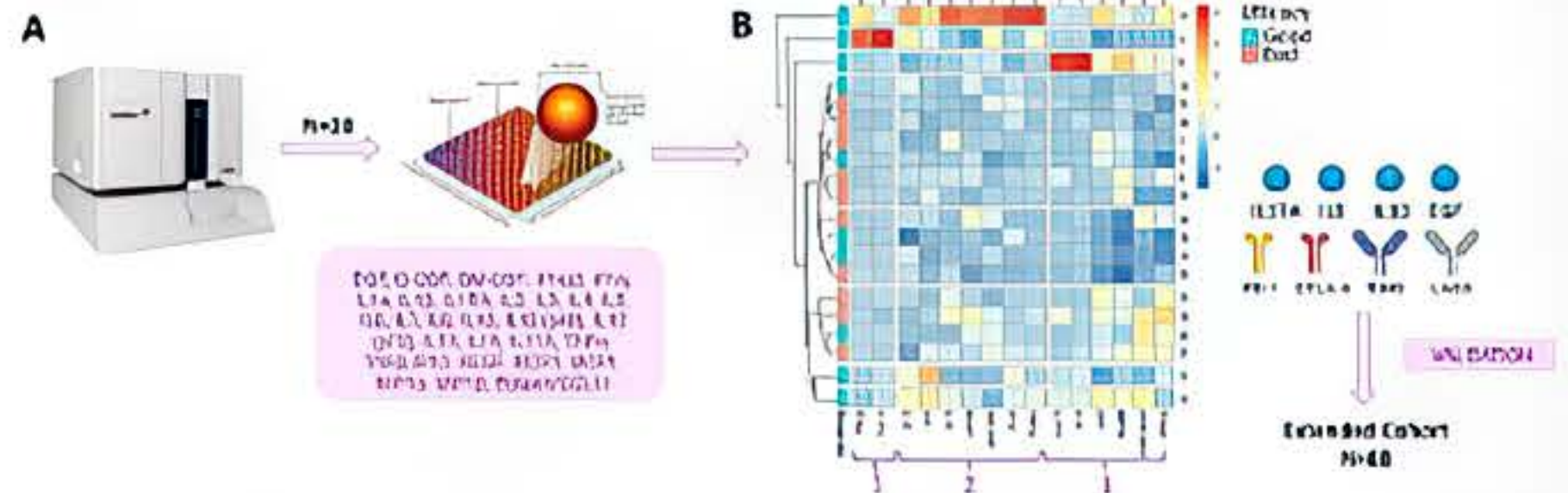


Figure 3. Evaluation of blood immune factors by Luminex®. (A) Schematic representation of the Luminex discovery assay employed to quantify 29 cytokines in a subcohort of 20 patients. (B) Heatmap of resulting cytokine clusters stratified by treatment efficacy using the Minkowski distance metric. The most informative cytokines from each cluster, along with selected soluble immune checkpoints, were further evaluated in an expanded cohort of 68 patients.

The most informative cytokines were further analyzed in the extended cohort, pre-treatment initiation. Although a trend towards higher expression in patients with reduced efficacy of CDK4/6i was observed, none of them were associated with treatment efficacy.

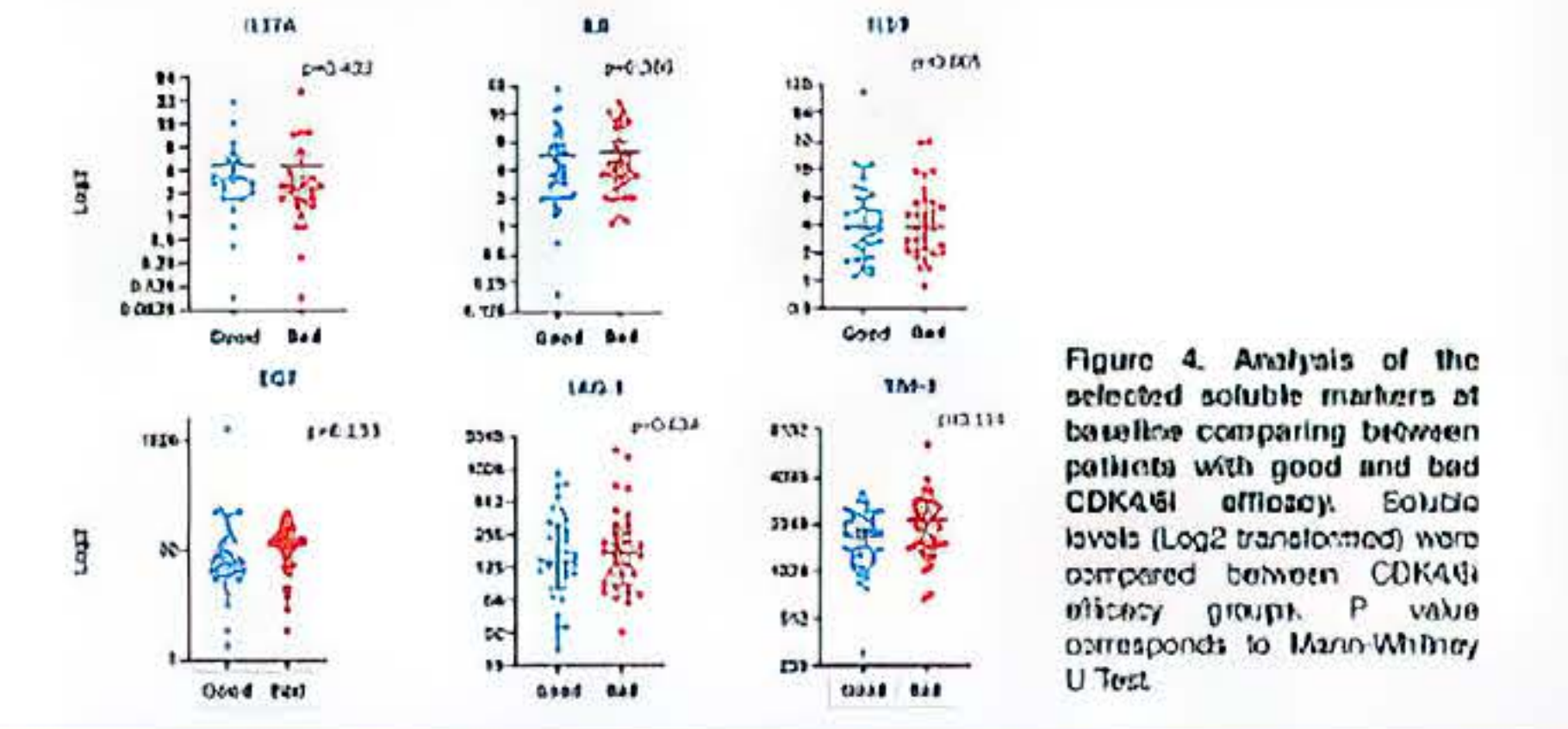


Figure 4. Analysis of the selected soluble markers at baseline comparing between patients with good and bad CDK4/6i efficacy. Soluble levels (Log2 transformed) were compared between CDK4/6i efficacy groups. P value corresponds to Mann-Whitney U Test.

Survival analysis of the longitudinal plasma samples at 3 different time-points



Figure 6. Study Sampling. Temporal distribution of plasma longitudinal samples in this project. N = 34.

In basal samples, low expression of TIM3 correlated with improved PFS (p=0.01) and OS (p=0.03). Longitudinal analysis showed the predictive value of IL8 at INT (p=0.043). A similar tendency for IL17A and LAG3 was observed, where low levels were associated with better PFS.

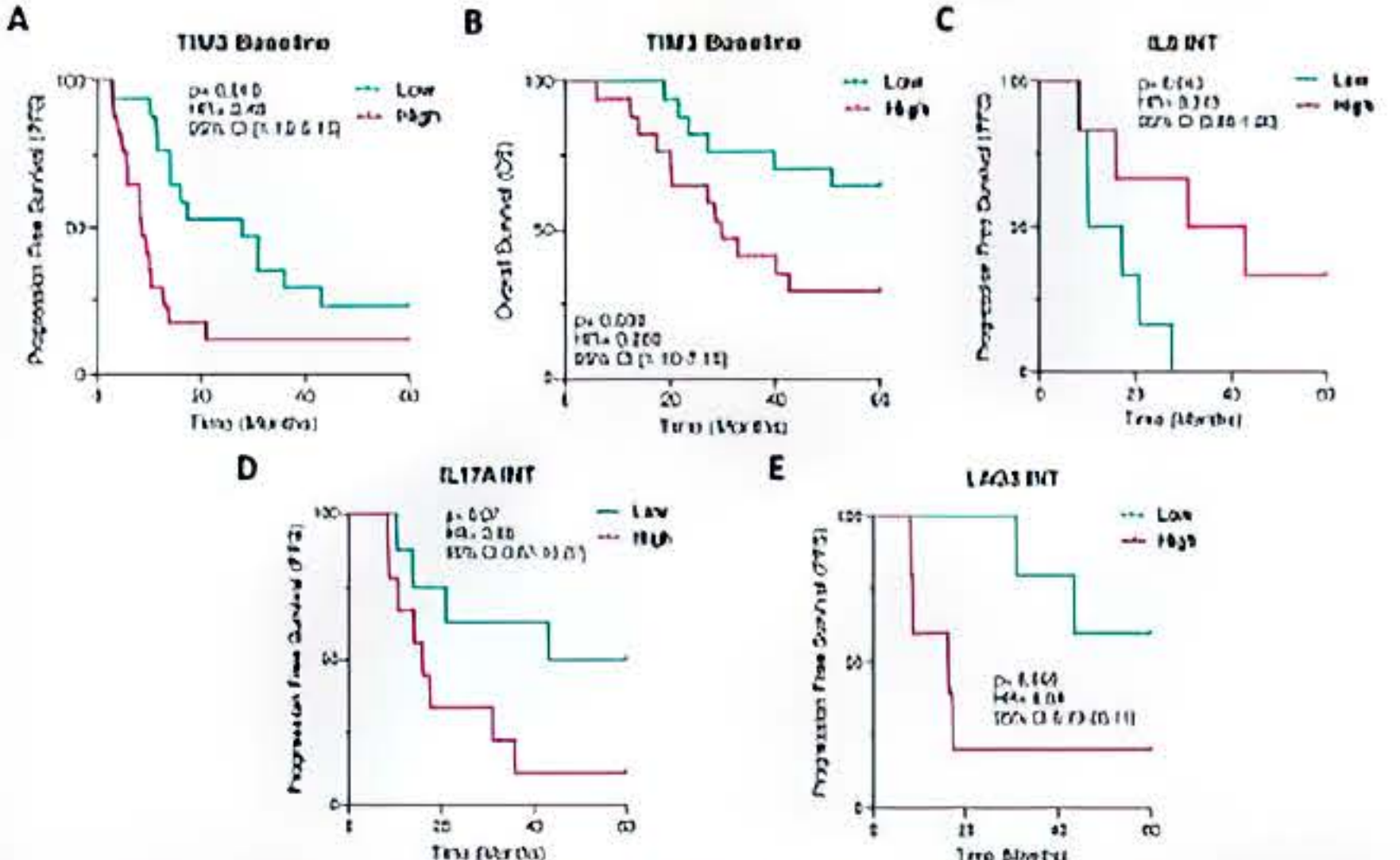


Figure 7. Kaplan-Meier survival curves. TIM3 at Baseline (A) PFS and (B) OS; IL8, IL17A and LAG3 at INT (C, D and E) PFS of patients split according to the median values of the indicated CXCs at Baseline and INT time-points.

Palbociclib treatment induces IL8 expression in spheroids and patient organoids

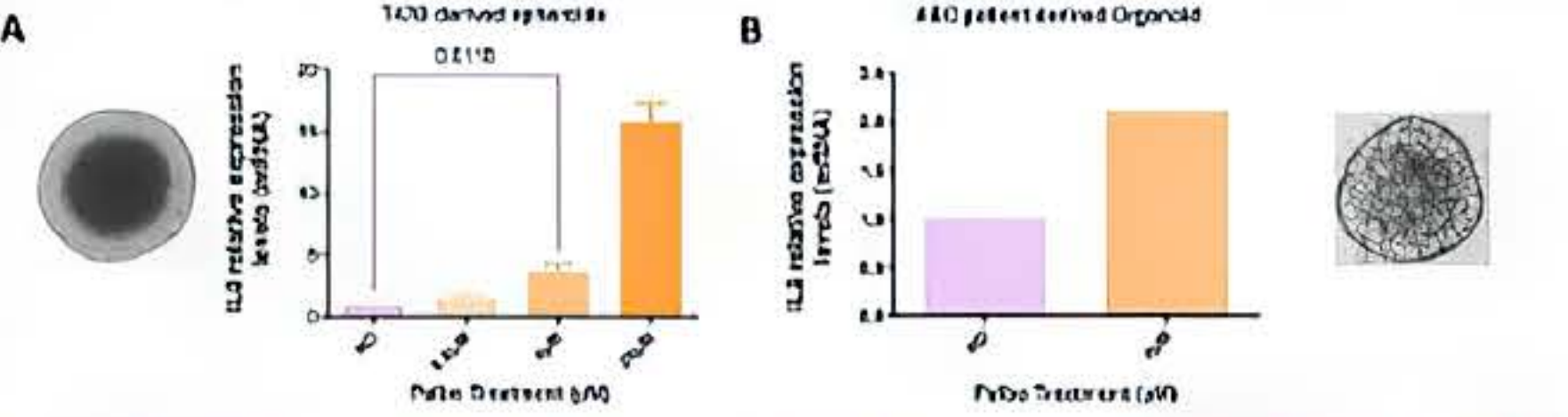


Figure 8. Relative mRNA and soluble (pg/mL) levels of IL8 in (A) T47D derived spheroids and (B) ABC patient derived organoid after Palbociclib treatment. T-Student p values were calculated relative to NonDrug condition in all cases.

Conclusions

- Immune function plays a key role in CDK4/6i treatment efficacy. Elevated inflammatory profiles within tumor but also in circulating immune cells are associated to limited CDK4/6i clinical efficacy, providing new tools for the clinical management of CDK4/6i treatment.
- Plasma levels of TIM3, IL8, IL17A, and LAG-3 may be reliable biomarkers for predicting CDK4/6i treatment efficacy in ABC.

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