Biomarker and multivariate analyses results from AIPAC: A phase IIb study comparing eftilagimod alpha (a soluble LAG-3 protein) to placebo in combination with weekly paclitaxel in HR$^+$ HER2$^-$ metastatic breast carcinoma.

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immutep LAG-3 IMMUNOTHERAPY

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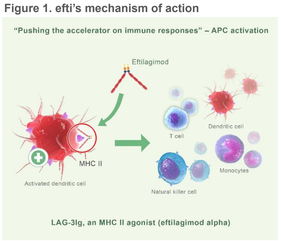
BACKGROUND

Figure 1. efti's mechanism of action



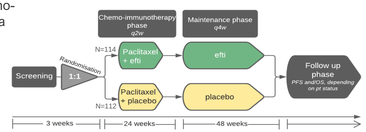
Eftilagimod alpha (efti) is a soluble LAG-3 protein targeting a subset of MHC class II molecules, thus mediating antigen presenting cell (APC) and CD8 T-cell activation (**Fig. 1**). This stimulation of the dendritic cell network and resulting T cell recruitment may lead to stronger anti-tumor responses in combination with paclitaxel than observed with paclitaxel alone. We report the final results from the randomized part of the AIPAC (Active Immunotherapy PAClitaxel *NCT02614833*) study in metastatic breast carcinoma (MBC) patients.

STUDY DESIGN & METHODS

Randomised subjects (using ECOG as the stratification factor) entered a chemo-immunotherapy phase followed by a maintenance phase (**Fig. 2**).

Subjects received 80 mg/m^2 paclitaxel intravenously days 1, 8, 15 plus efti/placebo subcutaneously days 2 and 16 up to 24 weeks and then efti/placebo s.c. every 4 wks.

Figure 2. Study design



Endpoints:

- **Primary Endpoint**: Progression-free survival (PFS) based on blinded independent central review (BICR) – RECIST1.1.
- **Secondary Endpoints**: Overall survival (OS), overall response rate (ORR), and others.
- **Exploratory Endpoints**: Pharmacodynamic (monocytes, CD4; CD8; CXCL10) biomarkers and their correlation with efficacy.
- Database cut-off date was May 14, 2021.

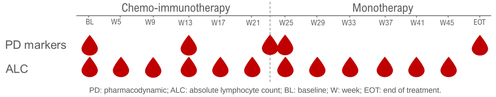
Multivariate Modelling:

- Poor prognostic and predictive markers using baseline characteristics were analysed in a Cox model using backward selection.
- The modelling strategy used backward selection with factors being excluded if p<0.15, including stratification factors as covariates.
- Final prognostic model was used for predictive model.

Pharmacodynamic Biomarkers:

- PD markers such as monocytes (CD45$^+$ CD14$^+$), activated CD4 (CD45$^+$ CD3$^+$ CD4$^+$ CD8$^-$ CD38$^+$ HLA-DR$^+$) or activated CD8 T cells (CD45$^+$ CD3$^+$ CD4$^-$ CD8$^+$ CD38$^+$ HLA-DR$^+$) were assessed by flow cytometry and plasma concentration of CXCL10/IP10 in a central lab according to the schedule in **Figure 3**.
- Absolute lymphocyte count (ALC) was measured locally at clinical sites acc. to **Fig. 3**.
- Samples were taken prior to the next efti/placebo administration to measure minimal residual effect.

Figure 3. Blood sampling schedule



PD: pharmacodynamic; ALC: absolute lymphocyte count; BL: baseline; W: week; EOT: end of treatment.

SUBJECT DISPOSITION

- All subjects were HR$^+$ and HER/neu$^-$ as per local assessment with majority as luminal B (49.1%) and luminal A (35.5%) as per central assessment.
- Subjects were heavily pre-treated with a median of 2 prior systemic anticancer regimens. Subjects were predominantly endocrine resistant (84%), while 44.2% were pre-treated with CDK4/6 inhibitors and received prior palliative therapy (74.8%). All subjects did not receive any prior chemotherapy for metastatic disease.

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PHARMACODYNAMIC BIOMARKER - RESULTS

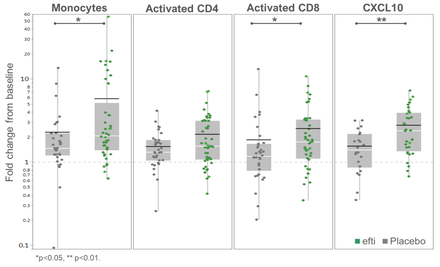
Analysis of fresh blood by FACS (subset of ~80 subjects)

- Efti significantly increased circulating levels of monocytes, CD8$^+$ T cells and CXCL10 compared to baseline (**Table 1**, **Fig. 4**). Increase of activated CD4$^+$ T cells was not significant.
- On-treatment increases are significantly linked to improved survival (overall median of 18.2 months used as a cut-off for "good" or "bad" OS) for subjects treated with efti, but not for subjects in the placebo arm except for activated CD4 (**Fig. 5**).
- Significant higher number of on-treatment circulating CD8$^+$ and CD4$^+$ T cells in subjects with improved survival in the efti group. For subjects treated with placebo, no effect observed or the change is not linked to improved survival (**Fig. 6-7**).

Table 1. Fold change of biomarkers compared to baseline

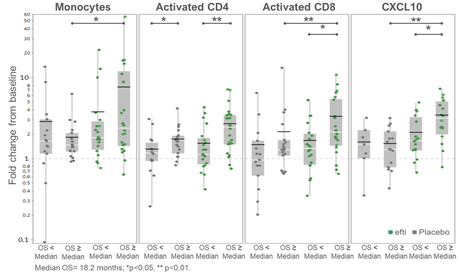
Biomarker	Treatment	Fold change mean ± SEM Median (25%Q-75%Q) [Min-Max]	p-value (2-sided rank-sum Wilcoxon test)
Monocytes	efti (n=42)	**5.81** ±1.49 **2.07** (1.40-5.16) [0.63-56.00]	0.025
	Placebo (n=34)	**2.29** ±0.44 **1.47** (1.21-2.23) [0.09-13.57]	
Activated CD4 T cells	efti (n=45)	**2.17** ±0.23 **1.56** (1.07-3.14) [0.42-7.13]	0.206
	Placebo (n=35)	**1.54** ±0.13 **1.31** (1.05-1.84) [0.26-4.14]	
Activated CD8 T cells	efti (n=42)	**2.54** ±0.35 **1.76** (1.10-3.25) [0.35-10.75]	0.027
	Placebo (n=34)	**1.86** ±0.40 **1.17** (0.79-1.67) [0.20-13.14]	
CXCL10	efti (n=32)	**2.78** ±0.30 **2.39** (1.36-3.93) [0.67-7.25]	0.006
	Placebo (n=22)	**1.56** ±0.18 **1.40** (0.86-2.18) [0.35-3.17]	

Figure 4. Fold change from baseline per biomarker



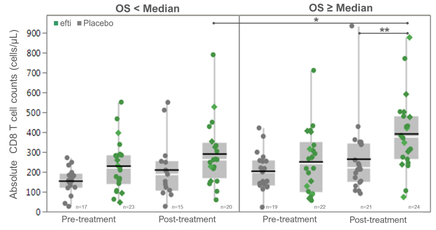
*p<0.05, ** p<0.01.

Figure 5. PD biomarkers by OS status



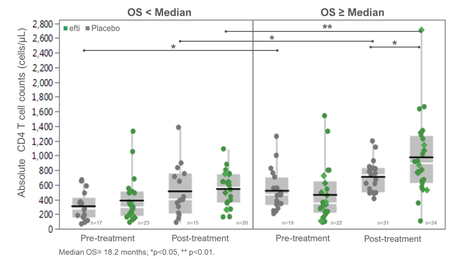
Median OS= 18.2 months; *p<0.05, ** p<0.01.

Figure 6. Absolute cell count of CD8 T cells pre- and post-treatment by OS status



Median OS= 18.2 months; *p<0.05, ** p<0.01.

Figure 7. Absolute cell count of CD4 T cells pre- and post-treatment by OS status



Median OS= 18.2 months; *p<0.05, ** p<0.01.

Analysis of general immune biomarker: Overall population

- Absolute lymphocyte count (ALC) showed early and sustainable increase within the efti arm. Effect is larger during maintenance phase after paclitaxel is stopped (after 6 months) (**Fig. 8 & 9**).
- Increase of ALC is linked to improved survival for subjects treated with efti arm but not for subjects in the placebo arm (**Fig. 9**).

Figure 8. Change of absolute lymphocyte count (ALC) from baseline

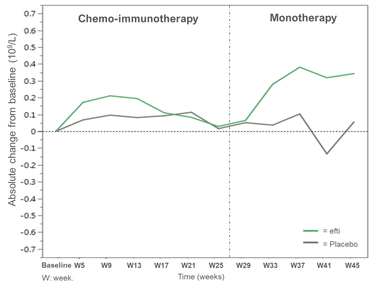
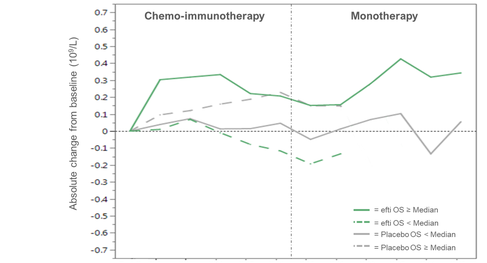


Figure 9. Change of absolute lymphocyte count (ALC) from baseline by OS status



Number of subjects											
Placebo OS ≥ Median (OS < Median)	52 (59)	51 (52)	48 (43)	43 (38)	38 (31)	38 (22)	35 (17)	33 (13)	23	18	12	11
efti OS ≥ Median (OS < Median)	60 (54)	58 (47)	54 (41)	53 (36)	49 (29)	42 (26)	38 (21)	37 (17)	26	21	17	10

W: week; Median OS= 18.2 months.
Note: visits only with ≥8 subjects displayed. Pre-dose at Day 1 of each Cycle

UNIVARIATE AND MULTIVARIATE ANALYSES RESULTS

Figure 10. OS - <65 years

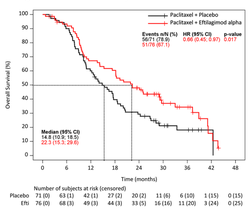


Figure 11. OS - Low monocytes

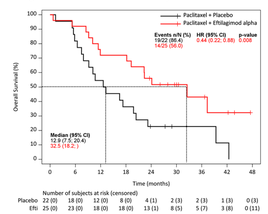


Figure 12. OS - No prior taxanes

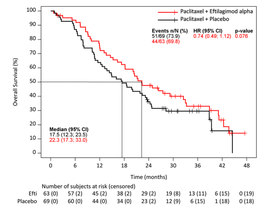
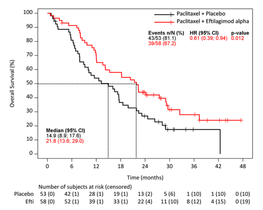


Figure 13. OS - High NLR ratio



NLR: neutrophil to lymphocyte

Figure 14. OS - <5 years since diagnosis

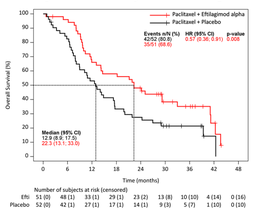
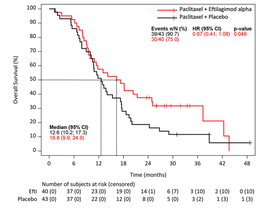
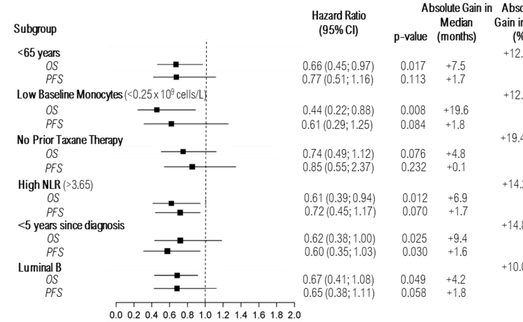


Figure 15. OS - Luminal B



- Through exploratory univariate analyses, six subgroups (**Fig. 10-16**) showed significant improvement in either OS, PFS and/or ORR compared to placebo.
- For overall survival (OS):
  - absolute gains ranged from 4.2 (luminal B) to 19.6 (low monocytes) months.
  - HR ranged from 0.44 (low monocytes) to 0.74 (no prior taxanes).
- For progression free survival (PFS):
  - absolute gains ranged from 0.1 (no prior taxanes) to 1.8 (luminal B and low monocytes) months.
  - HR ranged from 0.60 (<5 years since diagnosis) to 0.85 (no prior taxanes).
- For overall response rate (ORR) by BICR:
  - absolute gains ranged from 10.0% (luminal B) to 19.4% (no prior taxanes).

Figure 16. Forest plot of favorable (significant for ORR, PFS or OS) subgroups based on univariate analysis



PFS: progression-free survival by BICR (blinded independent central review); OS: overall survival; NLR: neutrophil lymphocyte ratio; ORR by BICR; *p=0.025; p-values not adapted for multiplicity.

MULTIVARIATE ANALYSIS RESULTS

- High BMI and prior CDK 4/6 were identified as independent poor prognostic markers significantly decreasing PFS (BMI: HR: 0.97, p=0.077; CDK4/6: HR: 1.65, p=0.001) and OS (BMI: HR: 0.97, p=0.041; CDK 4/6: HR: 1.37, p=0.072).
- Other factors were found to solely significantly decrease OS (months between primary diagnosis and IC [HR: 0.997, p= 0.015], high LDH (>250 U/L) at baseline [HR: 1.35; p=0.133) and high number (>2) of disease sites [HR: 1.65, p=0.008].
- When including the treatment effect, low monocytes and prior taxanes were found to be significant predictive factors for OS (p<0.15) (**Table 2**).

Table 2. Multivariate Analysis: Predictive markers for OS

Variable	OS Hazard ratio [95% CI]; p-value
Low monocytes	0.41 [0.16-1.03]; 0.005
Prior taxanes	0.85 [0.54-1.37]; 0.070

Note: Hazard ratios and p-values were estimated using a Cox proportional hazards model.

ECOG: Eastern Cooperative Oncology Group
FACS: fluorescence-activated cell sorting
RECIST: Response Evaluation Criteria in Solid Tumours

CONCLUSION

- Efti in combination with weekly paclitaxel significantly increases the number of primary (monocytes) and secondary (CD4 and CD8) target cells, which was not observed in the placebo group.
- The increase for these pharmacodynamic markers is significantly linked to improved overall survival in the efti group, but not in the placebo group.
- Absolute lymphocyte count (ALC) increases early and sustainably in the efti group and is linked to improved overall survival.
- ALC is a potential on-treatment predictive biomarker for efti.
- The multivariate prognostic model revealed relevant markers (e.g. prior CDK 4/6 treatment) to be used as stratification factors for future studies.
- Significant improvements in efficacy endpoints such as ORR, PFS and OS identified subpopulations in which efti may work better. This will be considered for patient population selection for future studies (e.g. phase III).
- Weekly paclitaxel + efti warrants further late stage development in HR$^+$ MBC.