12P-A gene expression profiling in early breast cancer (eBC) treated with neoadjuvant ribociclib plus letrozole (R+L) versus chemotherapy (CHT): A correlative analysis of the 1402-SOLTI/CORALLEEN phase 2 trial

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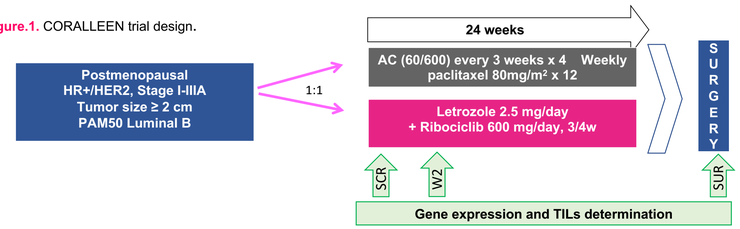
BACKGROUND AND OBJECTIVES

- The PAM50 Luminal B subtype represents ~30-40% of all hormone receptor positive (HR+)/HER2-negative (HER2-) eBC. (Cejalvo JM et al; *CTR*, 2018)
- The CORALLEEN was an open-label, parallel, phase II trial, randomized study in postmenopausal women with stage I-IIIA hormone receptor positive (HR+)/HER2-negative Luminal B breast cancer by PAM50. Patients (pts) received either 6 cycles of ribociclib plus letrozole (R+L) or 4 cycles of AC followed by 12 doses of paclitaxel (CHT arm).
- The primary endpoint was rate of PAM50 Risk of Relapse (ROR)-low disease at surgery. Similar response rates in the R+L arm and CHT arm were observed. (Prat et al. *Lancet Oncol*, 2019)
- We hypothesize that it is possible to identify biomarkers of response to CDK4/6 inhibitors and identify those patients with early PAM50 Luminal B breast cancer who can avoid chemotherapy.
- We present a comprehensive gene expression analysis done before, during (week 2 of treatment) and after treatment (at surgery) to molecular characterize the fully biology behind the primary results of the trial.

PATIENTS AND METHODS

- Formalin-fixed Paraffin-embedded (FFPE) tumor samples from the CORALLEEN trial (**Figure 1**) were obtained at screening (SCR), at week 2 of treatment (W2) and at surgery (SUR) from the two arms of treatment.
- Expression of 752 genes and 23 biological signatures were determined using the Breast360® panel by the nCounter platform (Nanostring Technologies, Seattle, USA). Stromal tumor infiltrating lymphocyte (sTILs) levels were assessed in the hematoxylin/eosin (H/E) sample. (Salgado R, et al. *Ann Oncol,* 2015)
- Response was defined as ROR-low disease at surgery, relative/absolute changes in ROR between baseline/W2 and surgery, residual cancer burden (RCB)-0/I or levels of ki67 at surgery.
- To identify genes associated with response a significance of microarrays (SAM) analysis with a false discovery rate (FDR) <5% was performed. ANOVA tests were done to observe differences in gene expression at different timepoints.
- All statistical analyses were carried out using the R software.

Figure.1. CORALLEEN trial design.



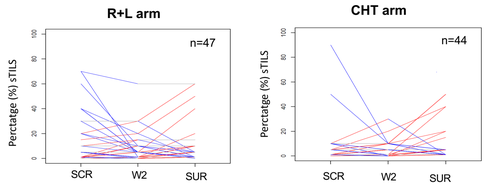
RESULTS

- A total of 298/307 (97.1%) of FFPE tumor samples were available for gene expression profiling (**Table 1**).
- No genes or signatures at SCR and W2 were found to be associated with response at surgery in neither arm.
- sTILs were not found to consistently increase/decrease at W2 or at SUR in either arms of treatment (**Figure 2**).

Table 1. Distribution of FFPE tumor samples accessible

Time point	R+L arm (samples = 149/153)	CHT arm (samples = 149/154)
SCR	51/52	51/52
W2	49/49	47/48
SUR	49/52	51/54

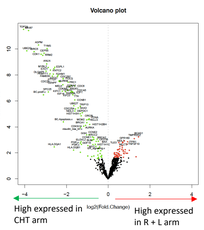
Figure 2. sTILs distribution in 3 timepoints between two arms of treatment



Different expression of genes and signatures between R+L arm versus CHT arm

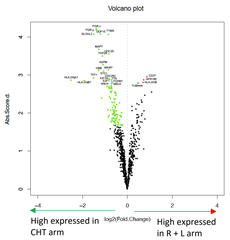
- At W2, 146 (18.8%) genes or signatures were significantly up-regulated (n=47) and down-regulated (n=99) in the R+L compared to CHT arm. (**Figure 3A**).
- R+L induced higher expression of genes related with DNA damage (e.g *TP53* and *RAD52*) and immune activation (e.g. *GZMM* and *CD19*) and lower expression of cell-cycle and hormone-related genes (e.g. *PGR*, *MKI67* and *CDK1*).
- At surgery 102 (13.2%) genes or signatures were found significantly up-regulated (n=4) and down-regulated (n=98) in the R+L arm compared to CHT arm (**Figure 3B**).
- R+L treatment induced higher down-regulation of estrogen and proliferation-related genes and signatures (e.g *PGR, ER* signaling and *MKI67)*.

Figure 3A. Dysregulated genes in R+L arm compared to CHT at W2.



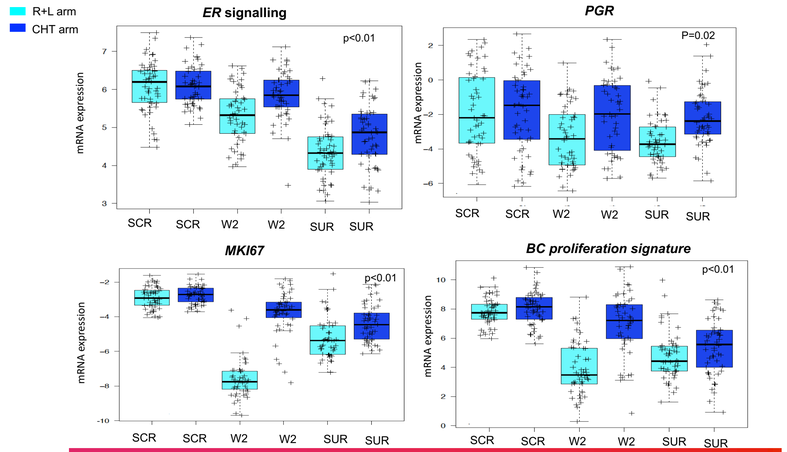
High expressed in R+L arm	High expressed in CHT
BCAS1	*TOP2A*
TMPRSS2	*MKI67*
GPR160	*ASMP*
ITPR1	*UBE2C*
NIEL1	*CDK1*

Figure 3B. Dysregulated genes in R+L arm compared to CHT at Surgery.



High expressed in R+L arm	High expressed in CHT arm
CD27	*PGR*
GPR160	*GDF15*
HLA.DO8	*MAPT*
TUBA4A	*CDC20*
-	*TOP2A*

R+L induced down-regulation of *ER sign., PGR, MKI67, proliferation* signature in W2 and surgery



CONCLUSIONS

- No genes or signatures were able to predict response in the R+L arm compared to CHT.
- Compared to CHT, R+L induced higher downregulation of proliferation and hormone-related genes/signatures at week 2 and surgery.
- These results support the strategy to use the neoadjuvant setting to select patients who achieve a larger molecular downstaging following ribociclib and endocrine therapy.

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