Treatment with ribociclib shows favourable immunomodulatory effects in patients with hormone receptor-positive breast cancer-findings from the RIBECCA trial


This is the final version of the accepted manuscript. The original article has been published in final edited form in:

European Journal of Cancer
2022 FEB; 162:45-55
2021 DEC 23 (first published online: final publication)
DOI: 10.1016/j.ejca.2021.11.025

Publisher: Elsevier
Treatment with ribociclib shows favourable immunomodulatory effects in patients with hormone receptor-positive breast cancer - findings from the RIBECCA trial

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KEYWORDS: CDK4/6 inhibitors; Ribociclib; Breast cancer; Immunomodulation; Adaptive immunity
Abstract

**Background:** Inhibitors of the cyclin-dependent kinases 4 and 6 (CDK4/6i) have significantly improved clinical outcomes in patients with advanced hormone receptor-positive (HR+) breast cancer and have demonstrated favourable antitumour immune responses in preclinical studies.

**Methods:** Here, we investigated peripheral immune responses to ribociclib in patients with metastatic HR+ breast cancer as a preplanned exploratory subanalysis of the RIBECCA trial (NCT03096847). Peripheral blood mononuclear cells were subjected to immune cell profiling, gene expression analysis of immune-related signatures, and deep T cell receptor profiling before treatment started and after 12 weeks of treatment with ribociclib.

**Results:** Gene expression analysis revealed an upregulation of signatures associated with an activated adaptive immune system and a decrease in immunosuppressive cytokine signalling during treatment with ribociclib. Profiling of peripheral immune cell subpopulations showed a decrease in Treg cell frequencies, which was associated with treatment response. Furthermore, induction of CD4+ naive T cells could be seen, whereas effector and memory T cell populations remained largely unchanged. Correspondingly, T cell repertoire diversity remained mostly unchanged during treatment, although an increase in clonality could be observed in single patients.

**Conclusions:** We show that treatment with ribociclib has significant effects on the peripheral innate and adaptive immune response in patients with HR+ breast cancer. Our data suggest that these effects lead to an activation of an already existing immune response rather than a de novo induction and make a strong case for future combination strategies of CDK4/6i with immunotherapies to enhance the adaptive immune response in HR breast cancer.

1. Introduction

Small molecule inhibitors of the cyclin-dependent kinases 4 and 6 (CDK4/6i), such as ribociclib, palbociclib and abemaciclib, have significantly improved progression-free survival (PFS) and, more importantly, overall survival (OS) in the MONALEESA-3 and MONARCH-2 trial in patients with hormone receptor-positive (HR+) metastatic breast cancer [1-3]. Inhibition of CDK4 and CDK6 results in loss of phosphorylation of the retinoblastoma tumour suppressor protein, thus cell cycle transition from G1 phase to S phase is blocked, and cell cycle arrest is induced [4].

As in most targeted therapies, tumours develop resistance to CDK4/6i over time [5]. Immunotherapy, on the other hand, has the potential to induce long-term remissions [6]. However, HR+ breast cancers are generally considered poorly immunogenic. They are characterised by a chronic inflammatory, protumourigenic microenvironment that can lead to a more aggressive, endocrine-resistant phenotype [7,8]. Immunosuppressive cell populations such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and M2 macrophages, as well as cytokines such as IL6, CCL22, TGFβ and TNFα are involved in this process [8]. This inflammatory microenvironment inhibits both T cell infiltration and T cell function and, thus, the induction of an effective adaptive immune response. Accordingly, the available limited data on immune checkpoint inhibition (ICI) in HR+ breast cancer showed only modest activity [9,10]. Neither did the combination of ICI therapy with chemotherapy improve response rates nor PFS [11]. Current strategies aim to develop combination therapies with effects on the immunosuppressive tumour microenvironment, which would be of special interest in endocrine-resistant tumours. CDK4/6i seem to be particularly interesting combination partners because not only do they have the potential to overcome resistance to antihormonal therapy, but they also show various favourable immunomodulatory properties in preclinical models [12].

CDK4/6i enhance antitumour immunity by suppressing DNA methyltransferase gene 1 expression in tumour cells, which leads to reduced DNA methylation and thus increases the expression of endogenous retroviral genes. These endogenous retroviral-transcripts induce a viral mimicry response that leads to stimulation of interferon production and expression of interferon-sensitive genes, including major histocompatibility complex class I genes, enhancing antigen presentation and genes involved in lymphocyte adhesion and co-stimulation [13]. Correspondingly, it has been shown in mouse models that CDK4/6i leads to an increase in intratumoural cytotoxic T cells and an inflamed tumour phenotype [13-15]. Moreover, CDK4/6i has shown inhibitory effects on immunosuppressive, protumourigenic cell populations such as CD11c+ myeloid cells and Tregs [13,14], thereby further
promoting a tumour-independent CD8+ T cell-mediated immune response [14].

Beyond data in mouse models, there are only limited data on the immunoregulatory effects of CDK4/6i in the more complex human immune system. Here, we analysed the influence of ribociclib on different peripheral immune cells and the T cell receptor (TCR) repertoire of peripheral T cells in patients with HR+ breast cancer treated with ribociclib plus letrozole within the RIBECCA trial (NCT03096847) and correlated the findings with patients’ treatment response and outcome.

2. Material and methods

2.1. Patients and blood samples

The analysis was carried out as a preplanned exploratory subanalysis of 40 patients, who have been treated within the RIBECCA trial (NCT03096847); a multi-centre, single-arm phase IIIb study to assess the efficacy and safety of ribociclib in combination with the aromatase inhibitor letrozole in patients with HR+, HER2- with metastatic or locally advanced breast cancer independent of menopausal status, as previously described [16]. Patients receiving steroids were excluded from the study. Radiological tumour assessment was performed using RECIST criteria version 1.1 every 12 weeks or when clinically indicated. Best response and clinical benefit were determined after 24 weeks of treatment. Investigation of prognostic and immunologic factors was approved by the Ethics Committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany (EC-ID: AZ 261_16), and patients had given informed consent before blood collection. Peripheral blood was collected before treatment started (week 0; W0) and after 12 weeks of treatment (W12) from which peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll Isopaque density gradient centrifugation and cryopreserved (University Hospital Erlangen, Erlangen, Germany). Of the 40 randomly selected patients from whom PBMCs were isolated, sufficient cell count and viability were available at both time points in 33 patients to perform multicolour staining; in addition, RNA isolation for gene expression analysis was possible in 30 patients. Of these, a randomly selected subgroup of 20 patient sample pairs was further used for TCR profiling.

2.2. Multicolour flow cytometry

PBMCs were stained using the DURACLone IM T Cell Subsets panel (Beckman Coulter, Brea, CA, USA) according to the manufacturer’s instructions. In 22 of the 33 samples, for which sufficient PBMCs could be isolated at both time points, the DURACLone IM Treg panel was applied additionally. Staining for HLA-DR (PerCP anti-human HLA-DR Antibody, BioLegend, San Diego, CA, USA) and CD11b (Brilliant Violet 605™ anti-human CD11b Antibody, BioLegend) was established in addition to the DURACLone IM Treg panel. Live/dead staining was performed using Zombie Red Fixable Viability Kit (BioLegend). The gating strategy is described in Supp. Fig. 1. Samples were acquired on a CytoFLEX S flow cytometer (Beckman Coulter), and data were analysed using FlowJo™ Software version 7.6.5.

2.3. Gene expression analysis

Total RNA was extracted from PBMCs using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA) and 4200 TapeStation system (Agilent Technologies, Santa Clara, USA) were used for quantification and measurement of RNA integrity. RNA was subjected to gene expression analysis using the CAR-T Characterisation Panel with the nCounter® Analysis System (NanoString Technologies, Seattle, WA, USA) following the manufacturer’s instructions. Differential gene expression analysis was performed using the R/Bioconductor software package limma [17] after background correction and data normalisation. P-values were adjusted to correct for multiple comparisons using the Benjamini-Hochberg (BH) correction. Gene set enrichment analysis was performed with the R-package fgsea [18].

2.4. Deep TCR profiling

To analyse changes in the TCR repertoire, deep TCR profiling was performed at the time points W0 and W12 of ribociclib treatment using next-generation sequencing. For this, cDNA was synthesised from isolated total RNA of patients’ PBMCs with the GoScript™ Reverse Transcriptase System (Promega, Madison, WI, USA), after which a TCRsafe™ analysis (HS Diagnostics, Berlin, Germany) was carried out to quantitatively analyse the TCRβ repertoire by next-generation sequencing as described previously [19].

2.5. Statistical analysis

Data analysis was performed with IBM SPSS Statistics software version 25 (IBM, Armonk, NY, USA) and figures were designed using GraphPad Prism version 9.1.2 (GraphPad Software, San Diego, CA, USA). For all analyses, a p-value < 0.05 (two-tailed) was defined as statistically significant.

3. Results and discussion

3.1. Patient characteristics and treatment response

Blood samples from patients with HR+ breast cancer receiving ribociclib in combination with letrozole were
analysed pairwise at W0 and W12 of treatment. An overview of the patient characteristics and treatment response of our subgroup of the RIBECCA trial is given in Table 1. All patients were female and had progressed to stage IV breast cancer at study entry. The overall response rate was 27.5% after 24 weeks of treatment. Compared to the full RIBECCA cohort of 502 patients, the clinical benefit rate at 24 weeks, which was the primary endpoint of the trial, was higher in our subgroup of randomly selected patients (87.5% versus 69.2%). This is clinical benefit rate at 24 weeks, which was the primary response of our subgroup of the RIBECCA trial is given overview of the patient characteristics and treatment response.

Table 1 Patient characteristics and treatment response.

<table>
<thead>
<tr>
<th>Patient and disease characteristics</th>
<th>N = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (IQR)</td>
<td>63 (53e67)</td>
</tr>
<tr>
<td>Menopausal status, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Premenopausal or perimenopausal</td>
<td>3 (7.5)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>37 (92.5)</td>
</tr>
<tr>
<td>Cohort A, no. (%)</td>
<td>30 (75.0%)</td>
</tr>
<tr>
<td>Cohort B, no. (%)</td>
<td>10 (25.0%)</td>
</tr>
<tr>
<td>Site of metastatic disease, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>28 (70.0%)</td>
</tr>
<tr>
<td>Bone only</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>Liver</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>Lung</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>Other</td>
<td>13 (32.5)</td>
</tr>
</tbody>
</table>

**Previous therapies**

- At least 1 prior antineoplastic therapy, no. (%) 29 (72.5%)
- Neoadjuvant as last therapy before study start 3 (7.5%)
- Adjuvant as last therapy before study start 18 (45.0%)
- Palliative treatment as last therapy before study start 8 (20.0%)

**Prior endocrine therapy, no. (%)**

- Treatment naive 13 (32.5)
- Previous endocrine therapy 27 (67.5)

**Prior chemotherapy, no. (%)**

- Treatment naive 19 (47.5)
- Previous chemotherapy 21 (52.5)

**Response to treatment**

- No. of patients with response (%) 11 (27.5%)
- Best overall response, no. (%) 1 (2.5)
- Complete response 1 (2.5)
- Partial response 10 (25)
- Stable disease 19 (47.5)
- Progressive disease 5 (12.5)
- Non-CR/Non-PD 5 (12.5)

- No. of patients with clinical benefit (%) 35 (87.5)
Table 2 Top 10 differentially expressed genes. Log2 fold change of mRNA expression levels of the top ten differentially expressed genes between pre-treatment (W0) and after 12 weeks of treatment with ribociclib and letrozole (W12).

<table>
<thead>
<tr>
<th>Gene</th>
<th>logFC</th>
<th>AveExpr</th>
<th>t</th>
<th>p-value</th>
<th>Adj. p-value</th>
<th>B</th>
<th>Annotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2L1</td>
<td>1.75</td>
<td>7.17</td>
<td>5.52</td>
<td>4.90855E-07</td>
<td>0.00039</td>
<td>5.68</td>
<td>Encodes BCL-XL (BCL-2 protein family), a potent cell death inhibitor</td>
</tr>
<tr>
<td>UBB</td>
<td>1.27</td>
<td>11.57</td>
<td>4.12</td>
<td>9.73956E-05</td>
<td>0.038966</td>
<td>1.16</td>
<td>Encodes ubiquitin, a key player in post-translational modification of various cellular proteins (ubiquitination)</td>
</tr>
<tr>
<td>TFDP1</td>
<td>0.85</td>
<td>6.30</td>
<td>3.72</td>
<td>0.000386174</td>
<td>0.102207</td>
<td>-0.01</td>
<td>Encodes TF that promotes E2F-dependent transcription in cell cycle progression</td>
</tr>
<tr>
<td>TRIM25</td>
<td>0.35</td>
<td>7.05</td>
<td>3.39</td>
<td>0.01122029</td>
<td>0.191273</td>
<td>-0.91</td>
<td>Encodes a TRIM protein family member involved in innate immune response</td>
</tr>
<tr>
<td>STAT1</td>
<td>1.05</td>
<td>8.60</td>
<td>3.37</td>
<td>0.00120449</td>
<td>0.191273</td>
<td>-0.97</td>
<td>Encodes a STAT protein family member, involved in the immune response to IFNs inducing a cellular antiviral state, JAK-STAT signaling pathway</td>
</tr>
<tr>
<td>IRF9</td>
<td>0.43</td>
<td>8.16</td>
<td>3.17</td>
<td>0.00220851</td>
<td>0.270096</td>
<td>-1.47</td>
<td>Enhances AKT1 and AKT2 in the PI3K-Akt signaling pathway</td>
</tr>
<tr>
<td>MTCP1</td>
<td>-0.52</td>
<td>4.10</td>
<td>-3.13</td>
<td>0.002552728</td>
<td>0.270096</td>
<td>-1.59</td>
<td>Encodes the anti-inflammatory cytokine IL11, which promotes MDSC differentiation via STAT3 signaling</td>
</tr>
<tr>
<td>IL11</td>
<td>-0.63</td>
<td>2.74</td>
<td>-3.09</td>
<td>0.002806737</td>
<td>0.270096</td>
<td>-1.67</td>
<td>Encodes the pleiotropic cytokine IL9, which is secreted by multiple types of immune cells, acts as a positive or negative immune regulator depending on cell type</td>
</tr>
<tr>
<td>IL9</td>
<td>-0.60</td>
<td>3.36</td>
<td>-3.06</td>
<td>0.003129965</td>
<td>0.270096</td>
<td>-1.76</td>
<td></td>
</tr>
<tr>
<td>MS4A2</td>
<td>-0.60</td>
<td>3.80</td>
<td>-3.03</td>
<td>0.003401711</td>
<td>0.270096</td>
<td>-1.83</td>
<td>Encodes the beta subunit of the IgE receptor involved in mast cell response</td>
</tr>
</tbody>
</table>

AveExpr: Expression mean for that gene across all samples; t: Ratio of the log2fold change to its standard error, which has been moderated across genes (moderated t-statistic); Adj. p-value: p-value adjusted for multiple testing to control the false discovery rate; B: Estimated log-odds probability ratio that the gene is differentially expressed; TF: Transcription factor; IFN: Interferon.

3.3. Treatment with ribociclib increased peripheral naive T cell frequencies

When analysing frequencies of peripheral T cell subsets during treatment with ribociclib, a significant increase in...
Fig. 2. Changes of frequencies in peripheral immune cell subsets during treatment.

Dots represent individual median frequencies of patients’ immune cell populations in PBMC before treatment (W0; indicated in red) compared to frequencies after 12 weeks of treatment with ribociclib and letrozole (W12; indicated in blue). Wilcoxon signed-rank test was applied to compare changes of cell frequencies between W0 and W12. (a) Median frequencies of CD4+ and CD8+ T cells were compared between W0 and W12 (CD4+ W0 66.2% [IQR, 51.8-75.3] versus W12 69.1% [IQR, 60.3-77.0], p = 0.006; CD8+ median decrease 6.8% [IQR, -16.9-3.6], p = 0.044); n = 33. (b), (c), (d), (e), (f) CD4+ and CD8+ T cells were further differentiated by their expression of CD45RA and CCR7 into naive T cells (CD45RA+CCR7+), central memory (CM; CD45RA-CCR7+), terminally differentiated effector memory (TEMRA; CD45RA+CCR7-) and effector memory (EM; CD45RA-CCR7-) T cells; n = 33. (b) Frequencies of CD4+ naive T cells significantly increased during treatment (median increase 42.7% [IQR, -6.7-157.2], p = 0.006). In the CD4+ naive T cell population, a significant increase in the early naive T cell subpopulation (CD27+CD28+) could be seen (median frequency W0 3.1% [IQR, 1.0-7.6] versus W12 5.2% [IQR, 1.8-7.8], p = 0.007). (g) A decrease of myeloid-derived suppressor cells (MDSC), defined as CD45+CD3-CD11b+HLA-DR- cells was seen during treatment (not significant); n = 22. (h) The ratio of effector (CD8+ T cells) to suppressor cells (CD4+FOXP3+CD25+) increased during treatment, however, not significantly. (i) A significant decrease in the T regulatory (Treg) cell population, defined as CD4+FOXP3+CD25+ cells, occurred during treatment (median frequency W0 1.2% [IQR, 0.8-1.7] versus W12 0.7 [IQR, 0.3-1.2], p = 0.007). This decrease was strongest in the Helios+ Treg subpopulation (median decrease 42.8% [IQR, 83.1-0.0], p < 0.001) but could also be confirmed in the CD4+CD25+CD39+ Treg subpopulation (p = 0.044); n = 22. PBMC, peripheral blood mononuclear cells.

circulating CD4+ T cells in the CD3+ population could be seen when compared pairwise to values before treatment (p = 0.006; Fig. 2a). Conversely, frequencies of total CD8+ T cells within the CD3+ population decreased slightly, resulting in a significant rise in the CD4+/CD8+ T cell ratio. We found that the increase of CD4+ T cells was mainly due to a significant expansion of the CD4+CD45RA+CCR7+ cell compartment, representing naive or antigen-inexperienced T cells with a median relative increase of 42.7% during treatment (p = 0.006; Fig. 2b). When further characterising the naive CD4+ T cell subpopulation within CD3+ cells, we saw that the expansion was mostly constituted by a CD4+CD45RA+CCR7+CD27+CD28+ subpopulation, representing early naive T cells [30] (p = 0.007; Fig. 2b). An increase in naive T cells was also seen in
Fig. 3. Association between changes in T cell frequencies and response to treatment.

Dot plots indicate percentage change of the respective cell frequencies during treatment with ribociclib and letrozole grouped by the response to treatment. Red bars represent mean and standard deviation. The Kruskal-Wallis test was applied for analysis. (a) No difference was seen in percentage change of CD8+ and CD4+ T cells between patients who responded to treatment (either complete response [CR] or partial response; n = 11) and those who did not (either progressive disease [PD] or stable disease; n = 19) or who achieved neither CR nor PD defined as Non-CR/Non-PD (n = 3). (b) No association between response to treatment and increase of CD4+ naive (CD45RA+CCR7+) and CD4+ early naive T cells (CD45RA+CCR7+CD27+CD28+) was observed. (c) Decrease in the CD4+FOXP3+CD25+Helios+ Treg cell population during treatment with ribociclib and letrozole was significantly associated with response to treatment (Kruskal-Wallis test p = 0.032). The largest decrease of Treg cell frequencies was seen in patients with response to treatment (n = 6) compared to patients who did not respond (n = 14) or patients who were defined as Non-CR/Non-PD (n = 2).

3.4. Development of T cell repertoire diversity and clonality during treatment with ribociclib

To investigate if the increase in circulating naive CD4+ T cells during treatment with ribociclib constitutes the initiation of a novel immune response, we performed
Fig. 4. Changes in T cell repertoire diversity and clonality during treatment.

Changes in diversity of the T cell receptor (TCR)β repertoire during treatment are represented as ratios of the Shannon diversity indices in W0 and W12 for each patient (grey). A ratio >1 indicates a diversification of the TCRβ repertoire during treatment, whereas a decrease in TCRβ repertoire diversity is expressed by a ratio <1. Differential clonotype development during treatment was assessed by comparing the frequency of identical CDR3 nucleotide sequences as ratios between W0 and W12 of treatment. Red squares represent mean ratios of the 20 most common clonotypes between W0 and W12 for each patient; standard deviations are depicted as upper error bars. The higher the value, the greater the clonotype expansion during treatment and vice versa.

TCR profiling in peripheral blood. The average number of individual TCRβ/CDR3 clonotypes with a frequency ≥0.01% was 1941 ± 1019 at W0 versus 2144 ± 739 in W12 of treatment with ribociclib plus letrozole (difference not significant). When comparing clonal diversification between W0 and W12 for each patient using the Shannon-Wiener diversity index, TCR diversity in the peripheral T cell repertoire remained largely unchanged (Fig. 4). However, when analysing differential clonotype development during treatment by comparing frequencies of identical CDR3 sequences between W0 and W12, we saw a significant rise in the median frequency of the 20 most common clonotypes between W0 and W12 in six of the 20 patients. In three patients, a significant decrease in clonality was seen, yet in two of the patients, this was accompanied by a gain in TCRβ diversity (Fig. 5). The clonal expansion was not associated with treatment response or PFS (data are not shown).

Hence, in our cohort, treatment with ribociclib plus letrozole seems to promote an already existing adaptive immune response rather than a de novo induction of an effector immune response. The antigen specificity of the expanded clones remains unclear. Few of them have been identified as public virus-specific clones, but it cannot be excluded that some of the pre-existing expanding T cell clones are indeed tumour-specific. More importantly, the possibility remains that a de novo adaptive immune response has been induced at the tumour site, which is not reflected in the peripheral blood. Tumour tissue sampling before and during therapy was not included as part of the protocol in this clinical study. Yet, the increase in circulating naive CD4+ T cells that we have observed during treatment with ribociclib could favour the initiation of a novel immune response, which just needs to be further amplified, e.g. by combination with ICI therapy [33]. Preclinical studies have demonstrated promising synergistic effects of CDK4/6i and PD-1/PD-L1 blockade [13-15]. First trials combining both mechanisms of action are currently ongoing in HR breast cancer and other tumour entities and seem to be feasible with respect to toxicity [34,35].

3.5. Treatment with ribociclib decreased peripheral immunosuppressive cell populations

In accordance with gene expression analyses, frequencies of peripheral regulatory T cells (Tregs, CD4+FOXP3+CD25+) significantly decreased during treatment with ribociclib plus letrozole (p = 0.007; Fig. 2i). In the CD4+FOXP3+CD25+ population, both, Helios and Helios- Treg subpopulations [36] declined significantly. This reduction was most pronounced in the Helios subpopulation, indicating a greater effect on a more activated Treg phenotype (Fig. 2i) [36].

Although not significantly, the ratio between CD8+ T cells and Tregs, the so-called effector to suppressor ratio (E:S ratio), increased during treatment compared to...
Fig. 5. Comparison of frequencies of the 20 most common TCR clones between W0 and W12 of treatment.
Box plots indicate median frequencies of the 20 most common identical clones in W0 and W12 for each patient; whiskers represent IQR. Clonotype frequencies were compared with the Wilcoxon signed-rank test. In 6/20 patients a significant clonal expansion was seen, whereas a significant decrease in clonality was seen in three patients.

values before treatment start, reflecting a change in the immune balance towards an enriched effector compartment ($p = 0.063$; Fig. 2h). Concomitantly, frequencies of peripheral MDSCs (CD45+CD3-HLADR-CD11B+) [37] decreased during treatment with ribociclib, however, not significantly ($p = 0.381$; Fig. 2g). As shown in previous studies, the suppression of proliferation of predominantly immunosuppressive cell populations such as Tregs and MDSCs during treatment with CDK4/6i seems to be relatively selective, while the proliferation of other T cell subsets is influenced to a lesser extent [13,38]. Tumour-infiltrating Treg frequencies have shown to be associated with poor clinical outcomes in HR+ breast cancer [39,40]. Interestingly, in our patient cohort, a decrease in the CD4+FOXP3+CD25+Helios+ Treg cell population during treatment was significantly associated with response to treatment ($p = 0.032$; Fig. 3c), however, did not translate into differences in patients’ outcomes. Associations of changes in Treg frequencies with clinical characteristics could not be observed (data are not shown).

4. Conclusions

In this comprehensive investigation, we demonstrated favourable and promising immunomodulatory effects of ribociclib in combination with letrozole in patients with HR breast cancer with regard to the innate and adaptive immune response. Although, the small samplesize and single time point of analysis during treatment must be considered limitations of the study that also rendered associations with clinical parameters exploratory. However, our data extend previous findings from preclinical mouse models to the human immune system and provide a strong rationale for combining or sequencing CDK4/6i such as ribociclib with immunotherapeutic approaches to enhance the adaptive immune response. If confirmed in a larger study, optimally with multiple time points of analysis during treatment, and in tumour tissue analyses, CDK4/6i may show to convert the immune-prohibitory tumour microenvironment in HR metastatic breast cancer to a more immune-permissive state and thus improve patient survival.

Ethics approval and consent to participate

The study was conducted as a preplanned analysis of the RIBECCA trial according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany (EC-ID: AZ 261_16). All subjects involved in the study had given written informed consent for trial participation and blood collection for this analysis.

Consent for publication

Written informed consent has been obtained from the patients to publish this paper.

Availability of data and material

Clinical data contains potentially personal information and is stored with the authors. Upon request, it can be shared in anonymised form with the editors or fellow scientists.

Funding

The research project was funded by Novartis Pharma GmbH. The funders had no involvement in the study design, in the collection, analysis and interpretation of the data, in the writing of the manuscript and in the decision to submit the paper for publication.
Authors contributions

CAP and AB designed, implemented and supervised studies, analysed and interpreted data and wrote the manuscript; SY implemented studies, acquired and analysed data and wrote the manuscript together with CAP and AB; CG, JK, SS acquired data; EG analysed data; SH analysed and interpreted data; IKN reviewed data and reviewed and revised the manuscript; DL and UK reviewed and revised the manuscript; SB, TD, PF, TF, WJ, SK, AS, MS and DL treated patients within the RIBECCA trial and are members of the RIBECCA trial consortium.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: SB, TD, PF, TF, WJ, SK, AS, MS and DL are members of the RIBECCA trial consortium and received speaker and advisory board honoraria from Novartis for work related to the RIBECCA study. CAP received speaker honoraria from Novartis for work unrelated to this manuscript. UK received speaker and advisory board honoraria from Novartis for work unrelated to this manuscript. The other authors declare no competing interests.

Acknowledgements

The authors thank Antonio Pezzutto for his critical discussion of the manuscript. In addition, the authors express their special thanks to Music Against Cancer e.V., which made this research project possible due to a grant, which helped finance personnel in the research group of Antonia Busse and Antonio Pezzutto at the Max-Delbrück-Center for Molecular Medicine in Berlin.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.11.025.

References


Supp. Fig. 1 – Gating strategy.
Representative dot plots of the gating strategy in PBMC of patients before and after 12 weeks of treatment. A First, lymphocytes were gated based on scatter profiles, doublets were excluded, and viable cells were gated based on viability stain exclusion (Zombie Red™), followed by gating CD3+ cells to identify T cells. In the CD3+ population, CD4+ and CD8+ T cells were discriminated. B Based on their expression of CD45RA and CCR7, subpopulations of central memory (CM), naive, terminally differentiated effector memory (TEMRA) and effector memory (EM) T cells were identified in the CD4+ and CD8+ population. Naive, CM, TEMRA and EM T cells were further subdivided into different stages of development by their expression of CD27 and CD28 into early (CD27+CD28+), intermediate (CD27+CD28-) and late subpopulations (CD27-CD28-). C T regulatory cells within the CD4+ population were estimated by their expression profiles of CD25, FOXP3, Helios and CD39. D T cells with an exhausted terminal effector phenotype (CD57+PD1+) were identified in the CD4+ and the CD8+ population. E To estimate frequencies of myeloid-derived suppressor cells (MDSC), first, monocytes were gated based on their scatter profiles, followed by discrimination of the CD45+ CD3- subpopulation, in which MDSC were identified by their expression of CD11b and lack of HLA-DR expression.

Supp. Fig. 2 – Change of frequencies in CD8+ naive and CD8+CD57+PD1+ T cells during treatment.
A An increase in naive CD8+ T cells (CD45RA+CCR7+) could be seen in the CD3+CD8+ population during treatment with ribociclib and letrozole (Wilcoxon signed-rank test, median frequency W0 2.2% [IQR, 0.4–5.2] vs. 2.4% [IQR, 0.9–6.2], p=0.044; n=33).
B No significant change was seen in patients’ CD8+CD57+PD1+ T cell population during treatment with ribociclib and letrozole (Wilcoxon signed-rank test, p=0.326; n=33).

Suppl. Table 1 – Leading-edge gene sets in gene set enrichment analysis.
Core genes of significantly up- or downregulated gene sets that accounted for the enrichment signal are indicated as leading-edge subsets.

<table>
<thead>
<tr>
<th>Name of gene set</th>
<th>Leading-edge gene subset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen processing and presentation</td>
<td>HLA-DQA1, SKP1, HLA-DR, PSMB10, HLA-E, RBX1, UBE2F, HLA-DRB1, IFI30, PSMA2, BATF3, UBE2V1, CYBB, PSMA6, UBA5, CDC6, CD14, PSMA3</td>
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<tr>
<td>Apoptosis</td>
<td>BCL2L1, TFDP1, PIK3R2, PSMB10, GZMB, NFkB1A, AKT2, PSMA2, STAT3, NMT1, TICAM1, PSMA6, CNTNB1, CD14, PSMA3, PIK3R1, CASP8, ADD1, CTSW, IL3RA, BCL2, GZMA, FOS, CTSD, PRF1, MAP2K2, BID, CASP3, PARP1</td>
</tr>
<tr>
<td>Autophagy</td>
<td>BCL2L1, PIK3R2, STK11, AKT2, WRAP45, CHMP4A, LAMP1, LAMP2, PIK3R1, HIF1A, TRAF6, BCL2, CHMP3, CTSD, MAP2K2, ATG14, MAP3K7, MTO1, RPTOR, AKT1, IGF1R</td>
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<tr>
<td>Cell cycle</td>
<td>TFDP1, SKP1, PSMB10, AKT2, RBX1, MAX, PSMA2, MYC, PSMA6, JAK2, CDC26, PSMA3, PRKCB, UBE2I, NACAPH, CKAP5, RAE1, SMG2, CDKN1A, CD7</td>
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<tr>
<td>Chemokine signaling</td>
<td>CCL2, CCL7, CXC11, CXC15, CCL21, CCL22, CXC113, CCL23, CCL25, CCL24, CX3C11, CCL26, CCL11, CCL19, CCL6, CCL3, CRK4, CCL8, CCL14, CCL16, CCL20, CXC6, GZMA, CCL1, CCL5, CCL15, CCL13, CCL6, CMKLR1</td>
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<td>Interleukin signaling</td>
<td>IL1R1, IL11, IL12, IL17F, IL12B, IL12, IL18, IL22, IL1B, RAG2, LIF, IL21, IL26, IL28B, IL28B, IL31, IL36RN, IL36, IL36RN, IL36, IL36, IL1RN, IL1RN, IL36G, IL36</td>
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<tr>
<td>Notch</td>
<td>STAT1, TFDP1, SKP1, PSMB10, GZMB, RBX1, PSMA2, MYC, PSMA6</td>
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<td>Oxidative phosphorylation</td>
<td>COX6B1, NDUFAB1, COX7A2, NDUF4, COX6C, COX7B, COX8B, COX11, ATP5MF, CS, NDUF4, COX5B, NDUF2A, LDHA, ATP5MG, PD3K, SDHB, MHC2, ID3H3A, UQCRQ, NDUF8B, COX16, NDUF6A, COX7C</td>
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<tr>
<td>TGFbeta</td>
<td>TFDP1, ID2, SKP1, RBX1, U5P15, MYC, NEDO8, TRIM33</td>
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<tr>
<td>Type I interferon signaling</td>
<td>IFIT3M, IFIT3, MX1, ADAR, HLA-E, IFI9, IFI9, IFI9, OAS2, STAT2, PTPN6, IRF4, OAS1, IFIT2, IFI6, XAF1, IFI35, ISG15, IFNAR1, OAS3, JAK1</td>
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<tr>
<td>Type II interferon signaling</td>
<td>MT2A, HLA-DQA1, HLA-DRB1, ICAM1, HLA-E, IFI9, TRIM22, TRIM22, TRIM21, IFI8, OAS2, TRIM10, HLA-DRB1, IFI30, TRIM25, IFN1R1, PTPN6, IFI4, IFNAR2, OAS1, JAK2</td>
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<td>Wnt signaling</td>
<td>UBB, SKP1, PSMB10, AKT2, RBX1, PSMA2, MYC, PSMA6, CNTNB1, RAC2, PSMA3, PRKCB, SMIARCA4, TCF7</td>
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