REPORT
25th SOCIAL RETURN OF THE RESEARCH
CANCER

IDENTIFICATION OF RESPONSE MECHANISMS TO CDK4/6 INHIBITORS IN HORMONE-RECEPTOR POSITIVE BREAST CANCER

Dr Aleix Prat Aparicio

IDIBAPS Institut d'Investigació Biomèdica August Pi i Sunyer

Dr Eva González Suárez

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

Dr Roger Gomis Cabré

IRBB Institut Recerca Biomèdica de Barcelona

1. Project summary

Hormone-receptor–positive (HR+) breast cancer is clinically and biologically heterogeneous, represents 75% of all breast tumors, and is a major cause of cancer death. Using gene expression analyses, we have shown that an important proportion of HR+ breast cancers do not fall into the luminal A or B subtypes but rather fall into the HER2-enriched (HER2-E) phenotype.

CDK4/6 inhibitors are approved for the treatment of HR+ metastatic breast cancer, significantly improving progression-free survival. Clinically, the luminal A and B subtypes show a substantial benefit from CDK4/6 inhibitors, while the HER2-E subtype is associated with low sensitivity to CDK4/6 inhibition and poor prognosis in both the early and the metastatic settings.

Our aim was 1) to identify molecular alterations behind resistance to endocrine and anti-CDK4/6 therapies as well as immune profiles, by analyzing up to 480 HR+ tumors from breast cancer patients treated with CDK4/6 in early-stage and metastatic settings; and 2) to identify biomarkers of drug response and treatment strategies, to overcome resistance to targeted therapies, using an unbiased CRISPR/Cas9 approach and in vitro and in vivo preclinical models of breast cancer.

We proposed a dynamic, translational approach starting from observations obtained in a clinical setting, moving to the lab to study them in depth, and finally returning to the clinic to test if the preclinical findings were also valid in the clinical setting.

2. Results

Combining molecular analysis of clinical samples and preclinical models, we have identified 11 key proteins involved in resistance to CDK4/6 inhibitors. In particular, we have focused on FGFR4, a membrane receptor that evades key complexes in the cell cycle (e.g. CCNE/CDK2) and that is a driver of the HER2-enriched phenotype. We have delved into the mechanism by which this membrane protein generates resistance, validated it in experimental preclinical models, and finally demonstrated as proof of concept that we can therapeutically modify the activity of this protein, thereby

improving tumor response to CDK4/6 inhibitors approved in clinical practice for HR+ breast cancer.

Additionally, a link between the RANK pathway and intrinsic/acquired resistance to CDK4/6 inhibitors has also been established. We found that RANK overexpression in HR+ breast cancer is associated with intrinsic resistance to CDK4/6i, both in vitro and in mouse xenografts, and decreased proliferation rate and chronic interferon (IFN) γ response are highlighted as resistance drivers. Gene expression data from clinical samples and studies with palbociclib-resistant cell lines, showed that RANK is upregulated after treatment with CDK4/6i, supporting a role in acquired resistance; and RANKL inhibitors can restore sensitivity to CDK4/6i and prevent acquired resistance.

Finally, we conducted shallow whole-genome sequencing on plasma samples from 459 metastatic breast cancer patients, including those treated with endocrine therapy and a CDK4/6 inhibitor. Our findings revealed that machine learning multi-gene signatures derived from copy-number detected in ctDNA can capture complex biological features relevant to tumor proliferation and estrogen receptor signaling. Importantly, we identified 4 DNA-based subtypes and a ctDNA-based genomic signature indicative of retinoblastoma loss of heterozygosity, both significantly associated with poor response and survival outcomes following treatment, regardless of plasma tumor fraction.

3. Relevance and potential impact

The consortium has concentrated its development efforts on HR+/HER2-negative tumors, the most prevalent breast cancer clinical subtype, representing 70% of cases. The introduction of CDK4/6 inhibitors to endocrine therapy has significantly improved the survival of patients with metastatic hormone receptor-positive and HER2-negative breast cancer. However, not all patients benefit equally, and eventually, all will experience relapse.

To address this, we need to develop new treatments for patients who progress on CDK4/6 inhibitors. Through the analysis of clinical samples and cellular models, we have identified FGFR4 as a potential resistance mechanism, which can be targeted

using a specific antibody-drug conjugate. These results may lead to the development of a new drug targeting FGFR4 including more preclinical validation and a first-in-human trial.

Furthermore, we conclude that pharmacological inhibition of the RANK pathway through RANKL blocking could represent an add-on to ET + CDK4/6i, warranting further clinical studies.

Additionally, we are working on developing biomarkers to help determine which patients will derive the most benefit from CDK4/6 inhibitors and which ones may require a different approach. We have identified a new biomarker based on DNA alterations that functions in both tumor tissue and blood samples.
We believe that the outcomes of this project could establish a new biological therapy paradigm, introducing novel therapeutic options a biomarkers for patients with metastatic HR+/HER2-negative CDK4/6i-resistant breast cancer.

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