TARGETING FGFR4 WITH AN ANTIBODY-DRUG CONJUGATE IN HORMONE RECEPTOR-POSITIVE AND HER2-NEGATIVE (HR+/HER2-) BREAST CANCER PROGRESSING TO CDK4/6 INHIBITORS AND ENDOCRINE THERAPY

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BACKGROUND

- Resistance to endocrine therapy (ET) and CDK4/6 inhibitors (CDK4/6i) is a major challenge in HR+/HER2- breast cancer (BC).
- The HER2-enriched (HER2-E) intrinsic subtype represents an aggressive molecular form of HR+/HER2- disease and is associated with significantly worse progression-free survival (PFS) and overall survival (OS) under ET+ CDK4/6i treatment compared to luminal subtypes (Prat *et al.* JCO 2021; CCR 2024).
- This work aims to better understand the underlying mechanisms driving ET+ CDK4/6i resistance and to develop new therapies.

RESULTS

- A genome-wide CRISPR/Cas9 loss-of-function screen was conducted in 2 HER2-E and palbociclib-resistant (PR) BC cell lines. Findings were integrated with gene expression data of tumor samples of patients treated with CDK4/6i in both early neoadjuvant (CORALLEEN trial [n=49 patients]) and metastatic (CDK cohort [n=375 patients]) settings. Eleven genes were identified as candidate resistance genes, including known mediators such as *CCNE1* (Fig. 1).
- Notably, *FGFR4* emerged as a novel and key driver of resistance (Fig. 1). *FGFR4* is a recognized driver of the HER2-E subtype in HR+/HER2-BC (Garcia-Recio *et al.* JNCI 2020, Hohmann *et al.* Nat Comm 2025).
- In the CDK cohort, the HER2-E subtype at baseline was associated with poor PFS (hazard ratio=3.04, 1.95–4.75, p<0.001) and OS (hazard ratio=3.09, 1.76–5.43, p<0.001). The prevalence of the HER2-E subtype increased from 10–20% at baseline to 40–50% in samples collected at progressive disease (PD) (Fig. 2).
- *FGFR4* was also associated with worse PFS and OS and it was increased in PD samples following ET+CDK4/6i (Fig. 3). Immunohistochemistry (IHC) analysis confirmed that FGFR4 protein levels strongly correlated with mRNA expression (r=0.66, p<0.001), were elevated in PD samples (Fig. 4), and were absent in healthy tissues (Fig. 5).
- Functional validation of FGFR4 was performed through knockdown and overexpression experiments in vitro, as well as in cell line-derived xenografts (CDX) and patient-derived xenografts (PDX). *FGFR4* knockdown reduced tumor growth, restored palbociclib sensitivity, and suppressed Cyclin E1 expression and RB phosphorylation. In contrast, *FGFR4* overexpression induced resistance and promoted HER2-E features (Fig. 6–8).
- To therapeutically target FGFR4, an antibody-drug conjugate (ADC) was developed, consisting of a humanized IgG1 antibody linked to the cytotoxic payload monomethyl auristatin E (MMAE) at a drug-to-antibody ratio (DAR) of 4 (Fig. 9).
- Unlike the naked monoclonal antibody, which showed limited in vitro activity, the FGFR4-ADC demonstrated high specificity, rapid internalization, and potent cytotoxicity in FGFR4-positive cells, while sparing FGFR4-negative cells (Fig. 9).
- The FGFR4-ADC also exhibited a bystander effect in co-culture experiments (Fig. 10) and significantly inhibited tumor growth in FGFR4-positive CDX and PDX models, with no detectable toxicities (Fig. 11).

Fig. 1. Integration of the translational analysis of two clinical cohorts and a genome-wide CRISPR/Cas9-based screen identifies 11 candidates of resistance.

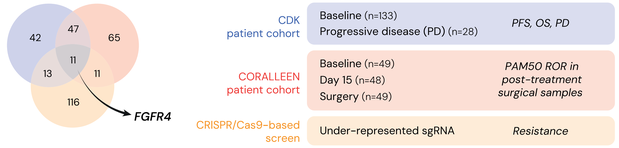


Fig. 2. PAM50 subtype distribution in baseline and PD samples of patients treated with ET+CDK4/6i.

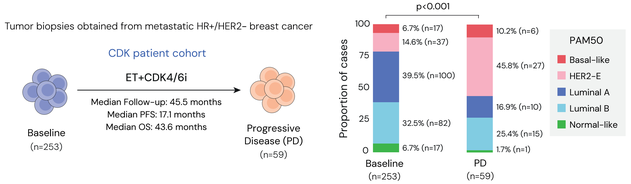


Fig. 3. High *FGFR4* mRNA expression is associated with poor PFS, OS and PD in the CDK patient cohort.

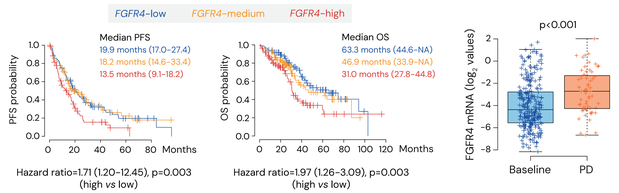


Fig. 4. FGFR4 protein expression correlates with *FGFR4* mRNA and it is increased in PD samples.

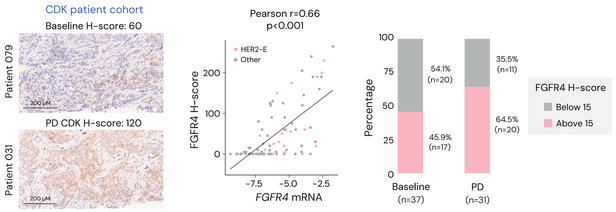
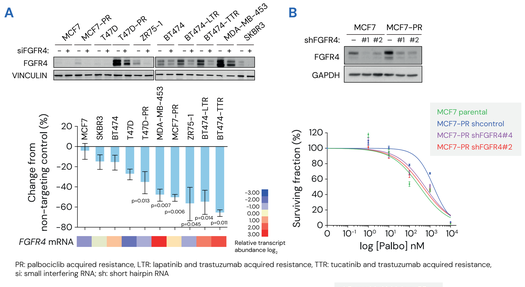


Fig. 5. Examples of FGFR4 protein expression in healthy tissue.

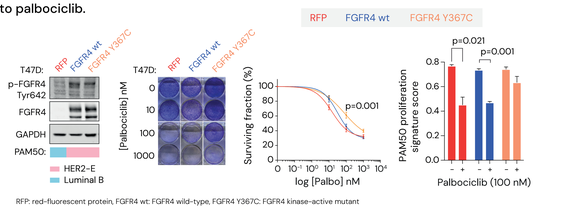


Fig. 6. *FGFR4* knockdown (A) reduces cell proliferation in cells resistant to palbociclib and (B) restores palbociclib sensitivity *in vitro* and (C) *in vivo*.



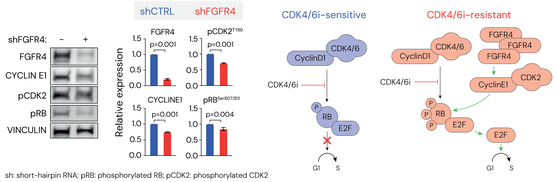
PR: palbociclib acquired resistance, LTR: lapatinib and trastuzumab acquired resistance, TTR: tucatinib and trastuzumab acquired resistance, si: small interfering RNA; sh: short hairpin RNA

Fig. 7. Overexpression of FGFR4 leads to a switch to the HER2-E subtype and resistance to palbociclib.



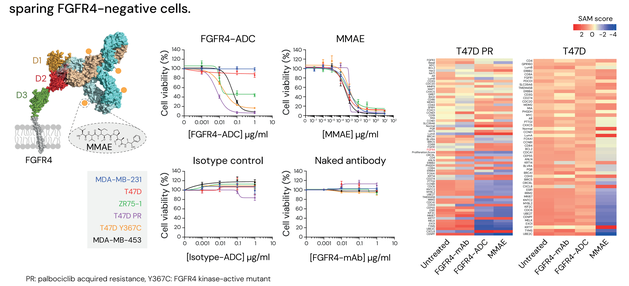
RFP: red-fluorescent protein, FGFR4 wt: FGFR4 wild-type, FGFR4 Y367C: FGFR4 kinase-active mutant

Fig. 8. FGFR4 regulates expression of Cyclin E1 and phosphorylation of CDK2 and RB.



sh: short-hairpin RNA; pRB: phosphorylated RB; pCDK2: phosphorylated CDK2

Fig. 9. FGFR4-ADC (MMAE payload) exhibits high specificity and cytotoxicity in FGFR4-positive cells, sparing FGFR4-negative cells.



PR: palbociclib acquired resistance, Y367C: FGFR4 kinase-active mutant

Fig. 10. The FGFR4-ADC has bystander effect in co-cultures of FGFR4-positive and FGFR4-negative cells.

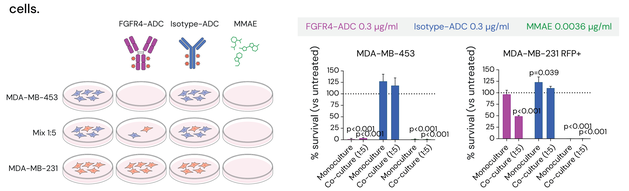
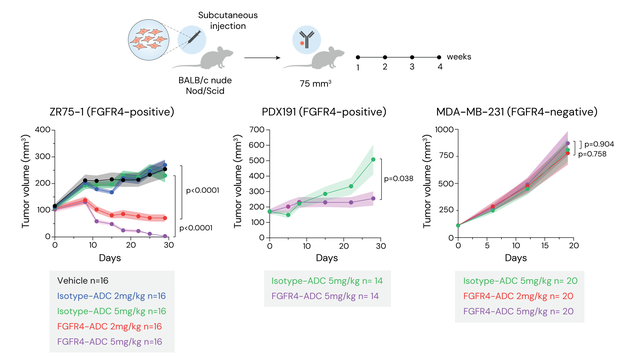


Fig. 11. The FGFR4-ADC inhibits tumor growth in CDXs and PDXs expressing FGFR4.



FGFR4 UPREGULATION DRIVES RESISTANCE TO ET+CDK4/6I IN HR+/HER2- BREAST CANCER.
A NOVEL FGFR4-ADC SHOWS STRONG PRECLINICAL EFFICACY.
AN OPTIMIZED FIRST-IN-CLASS FGFR4-ADC HAS BEEN DEVELOPED AND A FIRST-IN-HUMAN CLINICAL TRIAL IS EXPECTED TO START IN Q4/2025 IN SPAIN IN PATIENTS WITH ADVANCED HR+/HER2- BREAST CANCER.

Conflicts of interest of the first author: F.B-M. reports part-time employment from Reveal Genomics and has patents filed: PCT/EP2024/072737, PCT/EP2022/086493, PCT/EP2023/060810, EP23382703, and EP233833698.
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