Preclinical modelling of CDK4/6 inhibitor and Endocrine Therapy Resistance in ER+ Metastatic Breast Cancer using patient-derived xenografts

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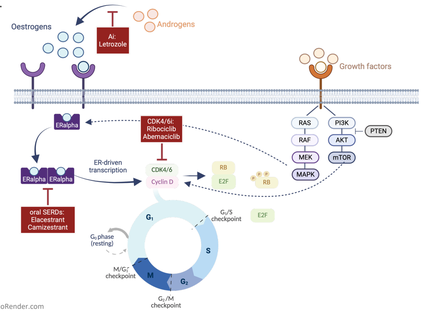
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1. BACKGROUND

Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in combination with endocrine therapy (ET) are the standard of care for patients with oestrogen receptor-positive (ER+) metastatic breast cancer (MBC). However, a significant proportion of patients either present intrinsic resistance or acquired resistance over time, limiting the clinical benefit of these treatments. Understanding the molecular mechanisms driving resistance and identifying effective second-line therapies remain major clinical challenges.



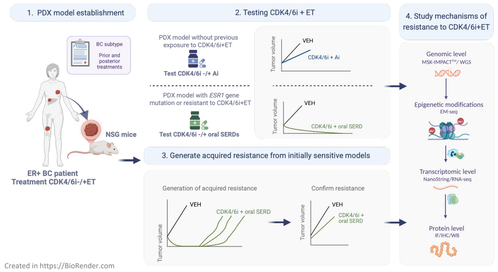
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2. AIM

AIM 1: To establish and characterize patient-derived xenograft (PDX) models from tumour biopsies collected from breast cancer patients before and after receiving CDK4/6i-/+ET.

AIM 2: To investigate mechanisms of intrinsic and acquired resistance to CDK4/6i+ET using genomic, epigenomic, transcriptomic and protein expression.

3. METHODOLOGY



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4. RESULTS

Figure 1. PDX models recapitulate molecular breast cancer patients' subtype

A total of 18 PDX models were successfully established from 17 breast cancer patients (**A**). PDX models accurately preserve the molecular subtype of the original breast cancer patient from whom they were derived (**B**).

A

Biopsies collected for PDX establishment		
Number of patients		16
Number of biopsies		17
Primary site	Breast	1/17 (6%)
Metastatic site	Breast	8/17 (47%)
	Skin	5/17 (29%)
	Liver	2/17 (12%)
	Others	1/17 (6%)
Patient Intrinsic subtype	Luminal B	15/17 (89%)
	Luminal A	1/17 (5,5%)
	HER2-enriched	1/17 (5,5%)
Patient CDK4/6i+ET exposure	Naive	6/17 (33%)
	Exposed	7/17 (39%)

B



Figure 2. Heterogeneous response to CDK4/6i+ET across 17 ER+ PDX models

17 PDX models were tested for their sensitivity to CDK4/6i with aromatase inhibitor (Ai) and/or oral selective oestrogen receptor degrader (SERDs) (**A**). Of those, 8 PDX models were classified as sensitive and 9 PDX models showed intrinsic resistance. Baseline characterization of these PDX models at the genomic (MSK-IMPACT panel), transcriptomic (RNA-seq) and protein (WB) levels revealed that intrinsically resistant models were enriched for cell cycle alterations - molecular features previously associated with resistance to CDK4/6i+ET (**B**).

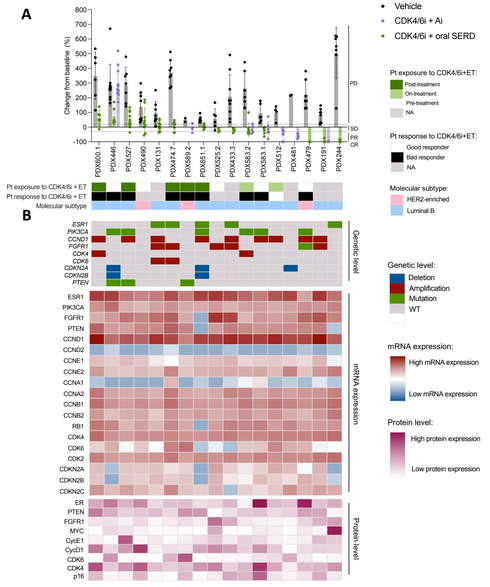


Figure 3. Generation of acquired resistance to CDK4/6i+ET in a sensitive PDX

In vivo efficacy of CDK4/6i (abemaciclib) and ET (elacestrant) was evaluated in PDX244 as single agents or in combination (**A**). The combination induced complete responses (CR). Tumours achieving CR were withdrawn from treatment until re-growth was observed. Upon re-treatment with the combination, tumours failed to respond and continued to grow, demonstrating acquired resistance. The 9 resistant tumours confirmed resistance to CDK4/6i+ET treatment (**B**).

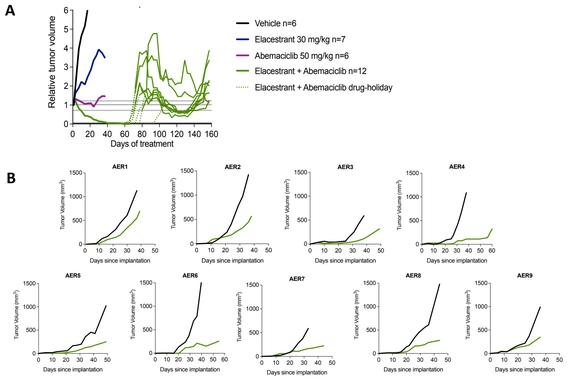


Figure 4. Characterization of acquired resistant tumours from PDX244

PAM50 analysis revealed no change in molecular subtype across the 9 resistant tumours with acquired resistance (**A**). Genomic profiling using the MSK-IMPACT panel did not identify any relevant mutation associated with resistance to CDK4/6i+ET. Protein expression analysis by WB revealed potential mechanisms of resistance, including CDK6 overexpression (AER2) or c-MYC overexpression (AER5 and AER7) (**B**).

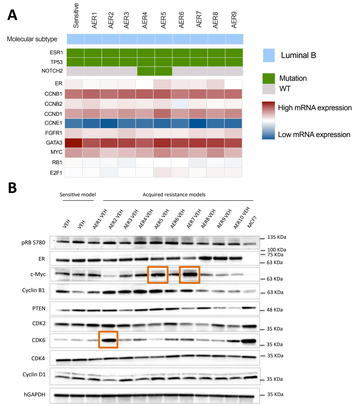


Figure 5. Short-term response of resistant PDX models

PK/PD experiments were conducted to evaluate the short-term response of resistant tumours to CDK4/6i+ET treatment over 12 days. The two resistant tumours (AER4 and AER8) exhibited an attenuated downmodulation of E2F1 and a concomitant rebound of CDK6 expression upon treatment compared to their sensitive tumours.

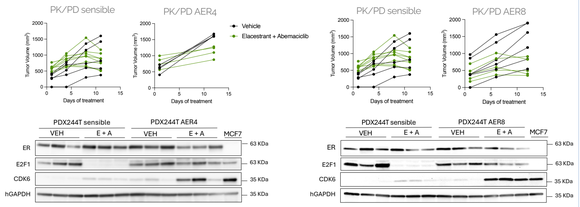
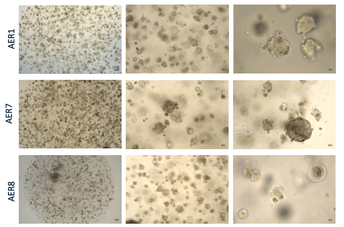


Figure 6. PDXO establishment for screening novel targeted therapies

Tumours with acquired resistance were grown as organoids to establish an *in vitro* platform for screening novel targeted therapies aimed at overcoming CDK4/6i+ET resistance.



5. CONCLUSIONS

In summary, our PDX collection provides a robust pre-clinical platform to study both intrinsic and acquired resistance to CDK4/6i+ET therapy. Importantly, not only comprises genomic mechanisms but also non-genetic layers of adaptation that may involve epigenetic reprogramming.

Ultimately, these models will allow us to identify new drivers of resistance and new vulnerabilities to develop personalised therapeutic strategies to improve HR+ BC patients' outcomes.

6. ACKNOWLEDGEMENTS

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