Introduction
HER2 is a receptor tyrosine kinase whose gene is amplified in ~20% human breast cancer patients. Allelic loss of the autophagy gene, beclin 1/BECN1, is associated with HER2 amplification in breast cancer; low beclin 1 mRNA expression is associated with increased risk of HER2-positive breast cancer; and overexpressed HER2 and Beclin 1 interact in cultured cells. However, the functional significance of HER2/Beclin 1 interaction and of altered autophagy in HER2-driven tumorigenesis and whether autophagy induction may be beneficial in preventing HER2-positive breast tumour growth is unknown.

Material and methods
We explored the regulation of autophagy in breast cancer cells by HER2 in vitro and the effects as well as genetic and pharmacological approaches to increase autophagy on HER2-driven breast cancer growth in vivo.

Results and discussions
Here we show that endogenous HER2 interacts with Beclin 1 in multiple HER2 +breast cancer cells and inhibits autophagy. Mice with a knock-in mutation in Beclin1 (Beclin1F121A) that leads to increased basal autophagy are protected from mammary tumorigenesis when crossed with mammary-specific HER2 transgenic mice, and HER2 fails to inhibit autophagy in primary cells derived from these mice.

Moreover, treatment of mice with HER2-positive human breast cancer xenografts with the Tat-Beclin 1 autophagy-inducing peptide inhibits tumour growth as effectively as a clinically used HER2 tyrosine kinase inhibitor (TKI). This inhibition of tumour growth is associated with a robust induction of autophagy, a disruption of HER2/Beclin 1 binding, and a transcriptional signature in the tumours that is distinct from that observed with HER2 TKI treatment.

Conclusion
These findings indicate that the HER2-mediated inhibition of Beclin 1 and autophagy likely contributes to HER2-mediated tumorigenesis. They also suggest that strategies to block HER2/Beclin 1 binding and/or increase autophagy may represent a new therapeutic approach for HER2-positive breast cancers.

Introduction
PTEN is one of the most frequently mutated tumour suppressor genes in cancers including triple negative breast cancer (TNBC). PTEN loss results in the aberrant activation of PI3K signalling and in sensitivity to inhibitors of key components of the pathway, such as p110β and AKT. Nevertheless, the benefit of PI3K pathway inhibitors (PI3Kpi) in the clinical setting has so far been modest.

We aimed to identify genes whose inhibition potentiates the effects of PI3Kpi on PTEN-deficient TNBCs.

Material and methods
We performed a genome-wide shRNA screening to identify genes that, when silenced, synergised with a p110β inhibitor, a AKT inhibitor or a pan-PI3K inhibitor in impairing the growth of MDA-MB-468 PTEN-null TNBC cell line.

We carried out a CRISPR-CAS9 screen on the top 144 hits to test whether their KO could suppress the phosphorylation of the downstream effector S6 in combination with PI3Kpi.

Results were validated using PTEN-deficient human cell lines with acquired resistance to PI3Kpi and a panel of TNBC cell lines with different PTEN status.

We also generated a mouse model in pure background in which the KO of pten and tp53 is restricted to the mammary glands to further characterise the synergisms in vivo.

Results and discussions
We identified a number of hits whose blockage enhanced the growth inhibition and the phospho-S6 suppression induced by PI3Kpi. The onset of acquired resistance also correlated with an impaired suppression of phospho-S6 by PI3Kpi, pointing at S6 phosphorylation as a marker of treatment response in PTEN-null tumours.

The screenings identified EGFR, that is frequently overexpressed/amplified in TNBC, and GNB2 and GNG5, which encode β and γ subunits of G protein.

The synergism between EGFR inhibition and PI3Kpi was validated in vitro by the use of approved drugs and the combination preferentially targeted PTEN-deficient TNBCs. Simultaneous inhibition of EGFR and p110β suppressed the growth of PTEN-deficient human tumour xenografts also in vivo with no sign of toxicity.

The KO of GNB2 sensitised to the activity of pan-PI3Ki, increased EGFR phosphorylation and enhanced sensitivity to anti-EGFR drugs, pheno-copying and amplifying the effect of p110β blockage.

Conclusion
We showed that EGFR and G proteins can orchestrate the sustained activation of the signalling downstream PI3K-AKT when PI3Kpi are applied to PTEN-null TNBCs. We envision that the identification of cues and GPCRs that act upstream G proteins and blunt the response to PI3K and EGFR inhibitors may unveil new therapeutic opportunities.

Symposium: Stem Cells

DEFINING THE CELL POPULATIONS RESPONSIBLE FOR SKIN CANCER INITIATION AND RELAPSE FOLLOWING THERAPY

A Sánchez-Danés*, C Blanpain. Université Libre de Bruxelles, Laboratory of Stem Cells and Cancer, Bruxelles, Belgium

Introduction
The identification of specific cell type from which cancer arises and the cancer cell population that resists upon therapy leading to tumour relapse constitute the main