The main thrust of our laboratory is to identify therapeutic strategies against KRAS mutant lung and pancreatic tumours. In recent years, inhibitors against KRAS oncoproteins selective for some of their mutations such as G12C and G12D, as well as pan-KRAS inhibitors active against all mutations, have been either approved by the FDA (sotorasib and adagrasib) or are undergoing clinical trials. Yet, their clinical efficacy is far from what was expected. In lung cancer patients, sotorasib does not increase overall survival compared to standard chemotherapy regimens due to the rapid appearance of tumour resistance.

We have used genetically engineered mouse models of lung and pancreatic tumours to compare the therapeutic efficacy of KRAS ablation with that of KRAS inhibition, and to interrogate the molecular mechanism responsible for tumour resistance. Whereas ablation of KRAS oncogenes eliminates both lung and pancreatic tumours completely with no signs of tumour resistance, KRAS inhibition results in the rapid appearance of resistance as previously observed in human tumours. We are currently exploring whether inhibiting KRAS signalling at independent nodes within its downstream or upstream signalling pathways, as well as in orthogonal pathways, will not only increase tumour responses but also prevent the appearance of tumour resistance.

"Increasing the efficacy of therapeutic strategies aimed at KRAS mutant lung and pancreatic tumours represents a major challenge for precision medicine in cancer."
Basic research diet for an additional month resulted in increased percentage KG12VloxPC2 tumour model. Extended exposure to the TMX tumour burden after 1 month of TMX exposure, reaching 64.9% Kras a continuous TMX-containing diet to mediate recombination bearing KloxC2 mice (n=21, 111 CT+ tumours) were subjected to of age. Once tumours were detected by CT analysis, tumour-CreERT2 driven genetically Kras-oncogene ablation is independent of the mutational burden of the tumours.

Pharmacological inhibition of oncolytic KRAS signalling induces rapid tumour resistance To study the effect of KRAS inhibition in GEM tumour models, we generated a Krasoncogene allele by replacing the G12V by a G12C mutation using homologous recombination in ES cells. Intratransfusion of Krasoncogene mice (Kras-Cre P) with Adeno-FLPs particles induced the development of lung adenocarcinomas indistinguishable from those previously observed in the Krasoncogene; Kras-Cre mice. Continuous exposure of these tumour-bearing mice to sotorasib for 1 month (100 mg/kg), a dose equivalent to that used in the CaseBreak100 clinical trial, resulted in the appearance of resistant tumours in all treated animals after 4 to 12 weeks of treatment. Resistant tumours did not differ from untreated controls, with the exception of the degree of apoptotic cells that remained elevated. Finally, resistant tumours displayed a clear trend towards higher histological grades.

Sotorasib-resistant tumours display amplification of the Krasoncogene allele and elevated levels of drug metabolism pathways To identify the mechanisms associated with resistance to sotorasib, we submitted resistant tumours to WES analysis. Interestingly, we did not identify amplifications in about 150 days of treatment and were subsequently submitted to WES analysis. Interestingly, we did not identify amplifications in its detoxification and reducing its effect in tumour cells. For further validation, we generated a Krasoncogene allele that is resistant to the drug. We found that the resistant tumours did not differ from untreated controls, with the exception of the degree of apoptotic cells that remained elevated. Finally, resistant tumours displayed a clear trend towards higher histological grades.

The therapeutic effect of Kras oncogene ablation is independent of the mutational burden of the tumours Unlike human lung tumours, Kras-driven genetically engineered mouse lung adenocarcinomas display few additional mutations. Hence, we interrogated the therapeutic effect of Kras oncogene ablation in tumours induced by uracilene, a chemical carcinogen known to induce hundreds of mutations. For this purpose, we used a Kras conditional (flxed) strain to which we added the CreERT2 loci described above. The resulting mice, Kras, were exposed to uracilene at 4 weeks of age. Once tumours were detected by CT analysis, tumour-bearing Kras mice (n=21, 111 CT+ tumours) were subjected to a continuous TMX-containing diet to mediate recombination of the Krasoncogene alleles. Kras ablation dramatically decreased tumour burden after 1 month of TMX exposure, reaching 64.9% of complete responses, a result similar to that observed in the Krasoncogene mice. Extended exposure to the TMX diet for an additional month resulted in increased percentage of those tumours undergoing complete responses (89%).

No progressive or stable disease was identified in this trial. Surviving Kras mice were allowed to live. More than half of them survived for 4 to 10 additional months. None of these mice displayed signs of tumour relapse. Mice sacrificed at human end points failed to reveal detectable lung tumours. These results indicate that the complete tumour regressions in the absence of tumour resistance observed upon KRAS ablation is independent of the mutational burden of the tumours.

To further characterise these sotorasib-resistant tumours, they were submitted to RNAseq analysis. Gene Set Enrichment Analysis (GSEA) of differential gene expression revealed upregulation of gene sets involved in the metabolism of drugs by cytochrome P450 (CYP450) and glutathione-S-transferases (GSTs), as well as proliferation-related pathways. These results suggest that resistance could, at least partially, emerge as a consequence of an altered metabolism of sotorasib, resulting in its detoxification and reducing its effect in tumour cells.