**Kras** mutant genetically engineered mouse models of human cancers are genomically heterogeneous

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**Kras** mutant tumors are largely recalcitrant to targeted therapies. Genetically engineered mouse models (GEMMs) of **Kras** mutant cancer recapitulate critical aspects of this disease and are widely used for preclinical validation of targets and therapies. Through comprehensive profiling of exomes and matched transcriptomes of >200 **Kras**G12D-initiated GEMM tumors from one lung and two pancreatic cancer models, we discover that significant intratumoral and interspecies variability among **Kras** mutant tumors is largely recalcitrant to targeted therapies.

**Mutations** in RAS have been found in >80% of cancer indications (1, 2) with the majority occurring in KRAS (3). These tumors are one of the largest unmet clinical needs in oncology. The foundation and advancement of our understanding of this disease subset is due to the vast experimentation utilizing **Kras** mutant genetically engineered mouse models (GEMMs). However, the models themselves are poorly characterized. For **Kras**G12D-initiated GEMM tumors, places them in context of human patients, and demonstrates how to exploit this inherent tumor heterogeneity to discover therapeutic vulnerabilities.

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**Results**

**Spontaneous Acquisition of Genomic Aberrations in KrasG12D-Initiated GEMM Tumors.** To better define the genomic landscape of established **Kras** mutant tumor models, we focused on three models of **Kras** mutant cancer: a nonsmall cell lung cancer (NSCLC) model with adenosarcoma-induced expression of **Kras**G12D and homozygous p53 targeting (KPR: **Kras**<sup>LSL.G12D<sub>wt</sub></sup>**<sup>p53<sub>fl/fl</sub></sup>) (11, 13), and two models of spontaneous pancreatic ductal adenocarcinoma (PDAC) initiated by Pdx1-driven recombinase expression in the developing pancreas to induce **Kras**G12D expression, with either heterozygous loss of p16-p19 (<sup>Cre</sup><sup>MDM2<sub>b</sub></sup>) in the presence of p52 mutation (KPR: **Kras**<sup>LSL.G12D<sub>wt</sub></sup>**<sup>p16<sub>fl/fl</sub></sup></sup><sup>p53<sub>LSL.R270H<sub>d</sub></sub></sup><sup>Pdx1.Cre<sub>b</sub></sup>) or with homozygous targeting of p16-p19 (<sup>Cre</sup><sup>MDM2<sub>b</sub></sup>) in the absence of p53 mutation (KPR: **Kras**<sup>LSL.G12D<sub>wt</sub></sup>**<sup>p16<sub>fl/fl</sub></sup></sup><sup>Pdx1.Cre<sub>b</sub></sup>) (11, 14) (Table S1 and Dataset S1).

**Significance**

**Kras** mutant cancers represent a large unmet clinical need. **Kras** mutant genetically engineered mouse models (GEMMs) of cancer recapitulate disease characteristics and are relied upon preclinically to validate targets and test therapies. Our integrative analysis of GEMM tumors revealed significantly evolved genetic heterogeneity, a common feature of human tumors that underlines therapeutic response. Moreover, interspecies comparative analyses showed the extent of gene-level fidelity between altered oncogenes and tumor suppressors. The genomic diversity represents an unrecognized opportunity to identify therapeutically susceptible genomic subsets preclinically. Moreover, this more-thorough understanding of the unappreciated complexity in these model systems ultimately allows for better interpretation and translatability of preclinical GEMM data for the benefit of cancer patients.

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Data deposition: All source code and genomic data for the GEMMs are available at research.pub.gene.com/Kras-mutant-GEMM.

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We analyzed the profiles of exomes and matched transcriptomes of 221 KrasG12D-initiated GEMM adenocarcinomas (Table 1). Protein-altering mutations (PAMs) were found in >95% of tumors, with 1,908 total PAMs and on an average of nine PAMs per tumor (Table 1 and Fig. S1A and Dataset S2). There were no recurrent hotspot mutations (Dataset S2). PAM load varied widely among tumors even when isolated from the same animal (Fig. 1A), implying independent mutational susceptibility despite the shared host. In addition, there was no significant difference in PAM load between the models regardless of p53 status (ANOVA, P = 0.94). Twenty percent of PAMs are expressed (Dataset S2), consistent with recent work in human genomics studies where RNA-seq-based confirmation of protein-altering somatic variants ranged from 19 to 38% (15–17). When comparing to patient tumors, we found that the mutational frequency in GEMM tumors is 7- to 10-fold lower than in human KRAS mutant pancreatic and lung adenocarcinoma (never smokers), respectively (Fig. 1B and C).

In contrast to PAMs, extensive and strongly recurrent patterns of copy number alterations (CNA) were seen in all three models (Fig. 1D and Fig. S1B), including several genes known to drive cancer by this mechanism (18). Focusing on regions of recurrent amplification or deletion (GISTIC2.0 q value <0.1; Datasets S3–S5), NSCLC KP GEMM tumors displayed the most abundant CNAs. Cancer genes in those recurrently altered regions are, for example, Met, Keap1, Smarca4, Pml, Als2cl, Sikt1, Gna11, and Akt1. Focal amplification of a region harboring Kras in PDAC KPP GEMM was the only prevalent event in that model (Fig. S1B). Indeed, cooperating genomic alterations are acquired during tumor development in Kras mutant models. However, distinct from human tumors, PAMs are rare in Kras mutant GEMM tumors and instead CNAs are prevalent.

Shared Prevalent Gene Alterations Between Murine and Human Tumors. To determine the fidelity of genes spontaneously altered in the GEMM tumors relative to human KRAS mutant tumors, we directly compared the alterations observed in the Kras mutant GEMM tumors to those in corresponding KRAS mutant human indications in an unbiased manner, focusing on genes that are significantly altered in either species as per GISTIC2.0 or MutSig2CV. Using the prevalence of genetic alterations (CNAs and mutations) per gene in Kras mutant tumors from both species (Fig. 1E and Datasets S3–S5), we found that the majority of all genes are altered in <20% of tumors from either species. Moreover, there is little enrichment of alterations within cancer genes, relative to all genes in both species. Exclusivity of gene alterations by species (≥20% prevalence in one and ≤1% in the other) was observed, although rare overall. SMAD4 is an example of a human exclusive genetic alteration in pancreatic cancer that is notably absent from both PDAC KPP and KPR GEMM tumors. In NSCLC KP, four cancer-related genes were observed to occur with ≥20% prevalence in both species, two of which are genes genetically engineered in the murine tumors, Kras and Trp53, and two are spontaneously acquired, Keap1 and Sikt1. Thus, despite the divergence of the type of genetic alteration, PAM vs. CNA in human and GEMM, respectively, the GEMM tumors acquire aberrations in genes frequently observed in KRAS mutant human tumors.

Intratumoral and Intertumoral Genomic Heterogeneity in Kras-Mutant GEMM Tumors. Although spontaneously acquired genetic alterations were found in all Kras-initiated GEMM tumors, we sought to interrogate their frequency within tumors as a means to gauge their contribution to tumorigenesis and maintenance. Most mutations observed in the GEMM tumors are clearly subclonal (Fig. 2A and S1 C–F) and private, strongly suggesting that these are unlikely driver events required for tumor progression. Because allelic imbalance at the Kras locus is reported to be critical for in vivo KrasG12D-initiated tumorigenesis (8, 19–21), we assessed the prevalence and clonality of alteration of the driving oncogene. We observed Kras CNAs in all KrasG12D-initiated GEMM tumors, but the extent and penetrance varied widely (Fig. 2B). Ninety-one percent (58/64) of PDAC KPP tumors had focal gain or amplification (≥4 copies) of Kras with 3–26 copies (38% had ≥6 copies). Amplification was predominantly of the mutant allele (Kolmogorov–Smirnov test, P < 2.2e–16; Fig. 2C). Fluorescent in situ hybridization (FISH) of Kras revealed amplification in 38–57% of cells within tumors, with individual amplified cells carrying 20–60 copies, illustrating a high degree of intertumoral and intratumoral heterogeneity (Fig. 2D and Fig. S2). PDAC KPR tumors, however, showed Kras gain in 23% (9/40) of tumors, due to broad gain of chromosome 6 in 20% (8/40) of tumors (Fig. S1B). Loss of the wild-type Kras allele was rare (3% in both PDAC KPP and KPR) (Fig. 2B). For NSCLC KP, 92% of tumors showed allelic imbalance of Kras, of which 90% (95/106) gained only a single copy of Kras and 4% lost the wild-type allele, consistent with previous reports (19) (Fig. 2B). Again, single-cell analysis of Kras copy number aberrations revealed significant intratumoral heterogeneity of Kras in NSCLC KP tumors (Fig. 2E). Such intratumoral heterogeneity was also observed following single-cell analysis of Keap1 in NSCLC KP tumors, where individual lesions harbor cells experiencing heterozygous or homozygous deletion of Keap1 (Fig. 2F). Together these data reveal allelic imbalance as a commonly shared occurrence across Kras-initiated GEMM tumors. Moreover, single-cell analysis reveals broad intratumoral genomic heterogeneity of observed aberrations irrespective of model.

Cooccurring Genomic Alterations in Murine and Human Kras Mutant NSCLC KP Tumors. The NSCLC KP tumors demonstrated the most diverse genomic landscape (Fig. 1 D and E) among the KrasG12D-initiated models. Given such complexity and heterogeneity, we sought to clarify which cooccurring events harbor functional relevance within these tumors. To this end, we utilized the matched transcriptomic data from each tumor to identify genes whose expression levels reflect the underlying copy number alterations. Among the 115 NSCLC KP tumors, 141 focal regions were significantly gained or lost when considering both prevalence and magnitude of aberration (Fig. 1D; GISTIC2.0, with 2,274 expressed genes residing in these regions. Only 19% (438/2,274) of these genes have significant congruent copy number and expression changes (Spearman correlation ≥ 0.3), suggesting that only some CNAs lead to detectable expression changes.

To better understand the impact of cooperating or recurrent genomic events in the NSCLC KP tumors and enable comparison with human tumors, we focused on established cancer genes (4, 22, 23) that are located in significantly altered CNA regions in NSCLC KP tumors with congruent expression changes (≥0.25) (27 genes; Dataset S6). In parallel, we obtained the most common reported events (both mutations and CNAs) in human The Cancer Genome Atlas (TCGA) lung adenocarcinoma (18). We found five commonalities: Met, Keap1, Smarca4, Nkx2-1, and Sikt1 (Fig. 3A). Several of these genes reside within the same

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**Table 1. Summary of mutations in KrasG12D GEMM tumors**

| NSCLC (KP) | PDAC (KPP) | PDAC (KPR) |
|-----------|------------|------------|
| Matched pairs analyzed | 115 | 66 | 40 |
| Median no. of all mutations | 16 | 14 | 18 |
| Average no. of PAM per tumor | 8.5 | 9 | 8.5 |
| Median no. of PAM per tumor | 6 | 5 | 6 |
| Average no. of PAM per Mb | 0.23 | 0.24 | 0.23 |
| Maximum no. of PAM | 34 | 140 | 53 |
| Minimum no. of PAM | 0 | 0 | 0 |
chromosomal band, calling into question their functional consequence. For example, Keap1 and Smarca4 are located on the same lost chromosomal band in mouse, in addition to two other cancer genes, Icam5 and Dnmt1. Congruent transcriptional change was evident only for Keap1 (correlation 0.48) and Smarca4 (0.54), but was insufficient to distinguish the putative contribution of each given their similarity in prevalence and expression. Interestingly, these genes were all located on the same chromosomal band in...
human (*KEAP1/SMARCA4/ICAM5/DNMT1* on 19p13.2), further prohibiting functional disentanglement in human and mouse with these data. Additionally, *Met* and *Kras* reside on the same chromosome, but only in mouse. We interrogated the potential causal role of *Met* in tumors experiencing both *Kras* and *Met* CNA. By focusing on the genes that reside between

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**Fig. 2.** Intratumoral and intertumoral genetic heterogeneity in *Kras*-mutant GEMM tumors. (A) Distribution of the variant allele frequency for all observed PAMs from regions that remain copy number neutral, by model. The median of variant allele frequency per model: PDAC KPP, 0.06; PDAC KPR, 0.08; NSCLC KP, 0.1. (B) *KrasG12D* allele frequency associated with *Kras* copy number reveals selective amplification of G12D allele. Rare loss of the wild-type allele was observed (green halo): KPP *n* = 63, KPR *n* = 40, KP *n* = 115. (C) The distribution of genomic reads with G12D relative to the total number of reads mapping to *Kras* in normal tissues (light blue) or in tumors (pink). (D) FISH image from a representative KPP PDAC tumor for *Kras* (red) and a control gene (green). (E) FISH image from KP NSCLC tumors for *Kras* (red) and a control gene (green). Insets demonstrate adjacent cells with divergent *Kras* gains (white arrows, *Kras*). (F) FISH from two representative KP NSCLC tumors for *Keap1* (red) and a control gene (green). Cells with heterozygous loss of *Keap1* (solid white circles) and cells with homozygous deletion of *Keap1* (dashed white circles) are observed within the same lesion.
Kras and Met on chromosome 6, we are able to delineate instances where Met CNA may be due to gain of the entire chromosome, from cases where Kras and Met appear to be amplified independently, suggesting that in some tumors Met may indeed be functionally relevant in the GEMM. Additional genes abrogated in human lung adenocarcinoma tumors and featured in ref. 18 were altered in NSCLC GEMM tumors, but did not meet our stringent criteria of significance and concurrent transcriptional impact, such as Egfr (25% gain), Braf (90% gain), Arid1a (7% loss), Tert (30% gain/amp), Ccne1 (9% gain), and Ccnd1 (9% gain) (Dataset S6). In summary, oncogenes and tumor suppressor genes, beyond those genetically engineered, are recurrently altered in established KrasG12D GEMM NSCLC tumors, and similar events are mirrored in KRAS-altered human tumors.

Stk11 and Keap1 are the most prevalently altered genes that spontaneously occur in the NSCLC KP GEMM tumors and are shared with NSCLC patient tumors (Fig. 1E). Both genes experience loss-of-function mutations and copy number loss in human tumors. Contrarily, these alterations manifest solely as CNA in mouse tumors (Fig. 3A), and significantly reduced gene expression is observable in GEMM tumors with Stk11 and Keap1 genomic loss (Fig. 3B and Fig. S3A). To further assess the functional relevance of these events in the murine tumors, we applied human lung-derived signatures of KEAP1 deficiency (P values as per Mann–Whitney) (24). See Fig. S3B for expression of individual signature genes in NSCLC GEMM tumors.

Fig. 3. Comparative analysis of genomic events and transcriptional landscapes between murine and human KRAS-altered NSCLC tumors. (A) Comparative analysis of the common alterations in KRAS mutant human and murine NSCLC tumors. Protein-altering mutations and copy number changes of established tumor suppressors and oncogenes are summarized by species. Gene and prevalence (fraction) within Kras-altered tumors are listed. (B) Transcript levels are significantly lower in tumors harboring Keap1 losses compared with tumors with two copies. (C) NSCLC GEMM tumors with Keap1 deletion demonstrated a significantly enhanced human lung-derived signature of KEAP1 deficiency (P values as per Mann–Whitney) (24). See Fig. S3B for expression of individual signature genes in NSCLC GEMM tumors.

**Cooccurring Genomic Alterations in Murine and Human Kras Mutant PDAC Tumors.** In contrast to the lung model, the PDAC KPP tumors developed with focal amplification of KrasG12D but no other recurrent PAMs or CNAs with residing cancer genes (Fig. S1B). The PDAC KPR model, which differs in Tp53 status (mutant in KPR) and Cdkn2a status (homozygous loss in KPP, heterozygous in KPR), also experiences Kras allelic imbalance, although to a lesser degree (Fig. 2B). In addition, the PDAC KPR tumors frequently demonstrated allelic imbalance at the Tp53 locus, either through loss of wild type or gain of the mutant allele in 35% (14/40) of tumors (Fig. S3E). Tumors with allelic imbalance of Tp53 were not mutually exclusive to those with Kras CNAs in PDAC KPR tumors (Fisher’s exact test, P = 0.70).
When comparing the most common genomic events in murine PDAC with human KRAS-altered PDAC, the engineered co-drivers Trp53 and Cdkn2A were also the most prominent in human (Fig. 1E), suggestive of the dominance of Kras and G1/S signaling in tumors from both species. Smad4 loss was not observed in murine tumors, although alterations were observed for other Tgfb family members (Datasets S7 and S8). Focal amplification of Kras was characteristic of PDAC GEMM tumors and is also seen in human Kras-altered PDAC, although in only 5% of human tumors. To identify cooccurring events that harbor functional relevance within the tumors, we used the same criteria to align transcriptional changes to CNA (cancer genes with significant CNA by GISTIC2.0, and correlation ≥0.3 between CNA and expression in GEMM tumors); however, this yielded no additional events beyond those engineered (Dataset S7 and S8). Together, these data indicate that the engineered alterations represent the most prevalent ones observed in human tumors and are sufficient for tumor initiation and development in PDAC.

Functional Impact of Kras Allelic Imbalance and Genomic Complexity. With this overview of Kras allelic imbalance and cooccurring genomic events in the Kras-induced GEMM tumors, we sought to better understand the potential functional consequence of Kras CNA in the context of other genomic alterations. To do so, we exploited the matched tumor genomic and transcriptional profiles. First, we observed that the number of copies of Kras correlated with Kras expression (Fig. S4A), and similarly, KrasG12D allele frequency correlated with expression of mutant Kras (Fig. 4A) across all tumor models. Although MAPK pathway target gene expression was elevated in all models relative to normal tissue (Fig. S4B), these genes were consistently higher in PDAC KPP tumors (Fig. 4B). Moreover, expression of the signature genes highly correlated with increased KrasG12D allele frequency in PDAC KPP tumors (Fig. 4C). Interestingly, focal KrasG12D amplification was the only CNA or PAM found in these PDAC KPP tumors. The rare PDAC KPP tumors lacking focal KrasG12D amplification demonstrated copy number gain of genes capable of MAPK pathway activation, including Pdgfra, Braf, and Ret, which may underlie the consistent overexpression of MAPK target genes in this model. Metabolic changes have also been linked to Kras mutant expression and allele frequency (25, 26). Correlations with metabolism signatures were only observed in PDAC KPP tumors. KrasG12D allele frequency in KPP correlates significantly, although moderately with increased

Fig. 4. Functional impact of Kras allelic imbalance and genomic complexity. (A) KrasG12D RNA allele fraction correlates with KrasG12D DNA allele frequency in PDAC KPP (Left), PDAC KPR (Center), and NSCLC tumors (Right); ****p < 0.0001. Pearson R shown. (B) Unsupervised clustering of MAPK target genes by tumors with model type overlaid. Log2 reads per kilobase of exon model per million (RPKM) expression data per gene is converted to a standard score. Blue indicates low-scaled expression, and yellow indicates high expression for each gene. (C) KrasG12D allele frequency is correlated with the MAPK expression signature in PDAC KPP tumors (in cyan, Spearman correlation 0.65, P = 4e-10) and to a lesser extent in PDAC KPR tumors (in red, 0.49, P = 0.001) and NSCLC tumors (in orange, 0.39, P = 4.3e-5). (D) Glycolytic activity as measured by the glycolysis/PPP expression signature is weakly associated with KrasG12D allele frequency in PDAC KPP (Spearman correlation 0.3, P = 0.009), and not associated in PDAC KPR (0.12, NS) and NSCLC (0.06, NS). NS, P > 0.05.

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glycolysis/pentose phosphate pathway expression, as well as decreased peroxisome proliferator-activated receptor alpha (PPARA) and amino acid pathway expression (Fig. 4D and Fig. S4C). In summary, correlations with *Kras* effector pathway signatures were strongest in PDAC KPP tumors where the primary genomic alteration is allelic imbalance of mutant *Kras*, in the absence of other recurrent PAM or CNA events. This suggests that *KrasG12D* mutation and CNA are sufficient to predict effector pathway enrichments in established tumors only when occurring in isolation of other events.

**Differential Dependence on Mutant Kras in PDAC vs. NSCLC Tumors Under Selective Pressure of MAPK Pathway Inhibition.** Given the pronounced divergence of cooccurring spontaneous genomic events between NSCLC KP and PDAC KPP tumors, despite strong similarity with respect to *Kras* allelic imbalance (91% and 92%, respectively), we sought to interrogate how the heterogeneous allelic imbalance of *Kras* within these models is impacted under applied selective pressure. The underlying hypothesis was that in the absence of further gene modification, as in PDAC KPP, tumors would remain dependent on initiating events; whereas, in the context of high cooccurring genomic diversity, as with the NSCLC KP tumors, the initiating event may not be as critical. To test this hypothesis, we subjected both the PDAC KPP and NSCLC KP models to therapeutic intervention with MAPK pathway inhibitor combination treatment (Fig. 5). MAPK pathway inhibition significantly improved overall survival in the PDAC KPP model increasing the median survival by 53% (>2 wk; Fig. 5A), demonstrating a clear dependence of this model on MAPK pathway signaling. Knowing that heterogeneous *Kras* CNAs characterize this model (Fig. 2B and D), we assessed *Kras* status in terminal tumors. Following continuous MAPK pathway inhibition, PDAC KPP tumors were less heterogeneous, harboring a marked enrichment of cells with high *Kras* amplification.

![Fig. 5. Differential enrichment of Kras in PDAC and NSCLC tumors following therapeutic selective pressure of MAPK pathway inhibition.](image-url)

(A) Combination of MAPK pathway inhibition with a MEK inhibitor [cobimetinib (32) at 5 mg/kg, PO, QD] and ERK inhibitor [GDC-0994 (33) at 60 mg/kg, PO, QD] significantly increased overall survival of KPP PDAC animals (Log rank, ***<i>P</i> = 0.0001). (B) FISH image from KPP PDAC before (Left) and after (Right) tumors for *Kras* (red) and a control gene (green). Four of seven tumors analyzed demonstrated dense *Kras* clusters indicative of high copy amplification per cell in 100% of tumor cells. (C) Ratio of *KrasG12D* relative to total *Kras* detected from genomic DNA of terminal tumors from experiment in A determined by droplet digital PCR (ddPCR) (Vehicle, *n* = 8; MAPKi combo, *n* = 6; Mann–Whitney *P* = 0.029). (D) MAPK gene signature derived from terminal tumors in A by Fluidigm (Vehicle, *n* = 8; MAPKi combo, *n* = 6; Mann–Whitney NS, not significant). (E) Combination of MAPK pathway inhibition significantly prolongs survival in NSCLC GEMM (Log rank, **<i>P</i> = 0.001). (F) Ratio of *KrasG12D* relative to total *Kras* detected from genomic DNA of terminal tumors from experiment in A determined by droplet digital PCR (Vehicle, *n* = 9; MAPKi combo, *n* = 11). (G) MAPK gene signature derived from terminal tumors by Fluidigm (Vehicle, *n* = 10; MAPKi combo, *n* = 13; Mann–Whitney ***<i>P</i> = 0.0006).
(Fig. 5B and Fig. S5A). Furthermore, tumors demonstrated a significant increase in the KrasG12D allele fraction (Fig. 5C). Exome sequencing confirmed that 83% of treated tumors had a KrasG12D allele frequency exceeding the 90th percentile of baseline tumors (Fig. S5B). Progressing tumors also harbored MAPK target gene expression equivalent to vehicle tumors, despite continued drug treatment (Fig. 5D).

In the case of the NSCLC model, MAPK pathway inhibition also significantly improved overall survival increasing the median survival by 56% (>6 wk; Fig. 5E), demonstrating similar dependence of this model on MAPK pathway signaling. Yet, in NSCLC KP terminal tumors selective pressure of drug treatment did not alter KrasG12D allele fraction (Fig. 5F). However, at progression, MAPK signaling is significantly lower in treated tumors relative to control tumors (Fig. 5G). Together, these results imply that pancreatic tumor cells with dramatically amplified Kras CNAs and high KrasG12D allele frequency have a selective advantage in the context of continuous, long-term MAPK pathway inhibition, while in NSCLC, mutant Kras does not.

Discussion
Past efforts to comprehensively define tumor genomes have revealed critical tumor dependencies that ultimately enabled the development of more effective therapeutics and treatments for patients. Preclinical experimentation guides the discovery and evaluation of therapeutic targets, making it vital to fully understand the extent and limits of their fidelity to human correlates. Through comprehensive genomic and transcriptomic analyses, we demonstrate that the widely used PDAC and NSCLC KrasG12D-initiated GEMM tumors harbor previously unrecognized genomic diversity. Established oncogenes and tumor suppressor genes, beyond those engineered, are mutated, as well as recurrently amplified and deleted in tumors. The cooccurring genomic events significantly alter tumor transcriptomes indicative of a functional consequence that likely explains the transcriptional heterogeneity among tumors, despite sharing the KrasG12D initiating event.

It is unclear how the engineered alleles themselves, as well as the timing of acquisition, impact the evolutionary trajectory and heterogeneity. Use of distinct recombinases (i.e., FRT, Cre, Dre) combined with inducible elements (i.e., CreER, FlpOER, DreER) allow for temporal segregation of allele recombination, and conceptually, such tools could be used to better model the stepwise neoplastic transformation underlying human cancer. While temporally segregated recombination experiments extend beyond the scope of this current study, we anticipate that such research will provide unequivocal insight into the causality of each oncogenic lesion in compound genotypes. Additionally, whether alternate or multiple cells of tumor origin influence the genomic and transcriptional diversity among tumors remains a viable hypothesis. Another critical question is how such heterogeneity, irrespective of the origin, influences the signaling dependency of the established tumors, especially when under selective pressure, such as drug treatment or gene deletion. For example, it was recently reported that Kras copy number gain directs metabolic reprogramming in NSCLC progression (25).

However, correlations with metabolism signatures were only observed in PDAC KPP tumors, despite the fact that 92% of KP NSCLC tumors show Kras allelic imbalance (Fig. 4D and Fig. S4C). It is plausible that metabolic rewiring that occurs early during tumor development is later masked due to further genetic evolution and complexity, as seen in the KP NSCLC tumors, whereas in the PDAC KPP tumors, the primary genomic alteration is allelic imbalance of mutant Kras, in the absence of other genetic events, so evidence of the correlation persists. Of course, direct testing of metabolic inhibitors would be necessary to determine the true relationship between Kras allelic imbalance, heterogeneity, and metabolic dependency in established tumors.

The intense subclonality and intertumoral and intratumoral heterogeneity of CNAs and PAMs, especially in the NSCLC KP tumors, confounds the possibility of testing “causality” for each genomic combination with respect to tumor maintenance. Such genomic diversity and subclonality has been recently acknowledged in human NSCLC as well (27, 28), emphasizing that identifying actionable drivers is challenging given such heterogeneity. In terms of the fidelity of GEMMs, the mutational burden is considerably less than their human tumor equivalents, consistent with previous reports of other GEMM tumors, which may compromise their utility in certain contexts. However, Kras mutant GEMM tumors, however, show abundant copy number alterations, some of which achieve the same functional outcome as mutations in human tumors, confirming the fidelity between altered genes and pathway signatures.

The diversity of the genomic landscape observed within these tumor models represents a previously unrecognized opportunity to potentially identify genomic subsets linked to therapeutic response or resistance. Using therapeutic inhibitors of the MAPK pathway, we observe that two models (NSCLC KP and PDAC KPP) respond to treatment; however, this selective pressure leads to distinct outcomes with regard to the susceptibility of Kras-mutant cells to treatment. Despite the differences in cooccurring genetic diversity between the two models, both models significantly responded to drug treatment, suggesting that both systems harbor MAPK-dependent tumors. We then exploited the therapy-induced lesions to assess the impact of MAPK inhibition as a selective pressure on the initiating oncogene Kras. We discovered that in the context of PDAC KPP, therapy-resistant tumors enrich for highly amplified Kras cells, strongly indicative of a continued dependence on the initiating event. In recently published work using a KrasG12D acute myeloid leukemia (AML) model, where mutant Kras cooperates with disease-initiating retroviral insertions, allelic imbalance of Kras was found to increase susceptibility to MAPK inhibition (29). In contrast to our findings, the AML-relapsing tumor cells were nonamplified. It is notable that the heterogeneity and range of amplification is significantly higher in the PDAC KPP tumors. Moreover, it is likely that with enough Kras amplification, targeted therapies are unable to reduce signaling below the biologically meaningful threshold for effect. However, in the NSCLC KP tumors, therapy-resistant tumors demonstrate no change in the frequency of the Kras allele, suggesting no selective advantage in this context. It is tempting to speculate that the decreased dependency on amplified Kras in the NSCLC GEMM tumors is likely reflective of altered dependency on the initiating oncogene in the context of such genomic diversity, providing an enhanced fitness advantage and evolutionary flexibility. The fact that genomic evolution is occurring in these models provides a unique lens for these experimental models. Moreover, a more-thorough understanding of this unappreciated complexity in the context of both positive and negative outcomes observed in these model systems will ultimately allow for better interpretation and translatability of preclinical GEMM data for the benefit of cancer patients.

Materials and Methods
GEMMs. We licensed mice from Tyler Jacks (Massachusetts Institute of Technology, Boston); Exelixis, Inc.; Anton Berns (Netherlands Cancer Institute, Amsterdam); and Andy Lowy (University of San Diego, San Diego) (13, 14, 30, 31). KPP and KPP mice were euthanized at median ages of 16.4 and 9 wk, respectively. One to three individual tumors, in addition to control tissue (muscle) for genomic analysis, were collected from each animal. NSCLC KP animals were intranasally infected with $5 \times 10^6$ infectious units of adenovirus-expressing FLPe-IREs-Cre at ~8 wk of age. These animals were euthanized between 25 and 33 wk of age, a time at which adenocarcinoma is present. All animals were monitored according to the Animal Care and Use Committee (ACUC) at Genentech, Inc. For in vivo dosing experimentation of models, animals were randomized into treatment cohorts by tumor measurement, with equal numbers of male and female animals, Cobimetinib (MEK inhibitor) (32) and
Fluorescence in situ hybridization (FISH) was performed on the NSCLC 304:1497 1), and inferred their significance 339:1546 4), amplification (CN 26:873 28:423 156:1298 |< Cancer Res 517:489 Nat Biotechnol 26:873 28:423 156:1298 |< Cancer Res 517:489 Nat Biotechnol 149:656 110:113 42:404 110:113 42:404 110:113.

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