PANCREATIC CANCER

Cancer interception with KRAS inhibitors in preclinical models of pancreatic ductal adenocarcinoma

Minh T. Than[1,2], Lucie Dequiedt[3], Rina Sor[2], Shreya Nair[1,2], Nune Markosyan[1,2], Emma E. Furth[4], Chenghua Yang[2], Courtney Ray-Fofana[2], Marie Menard[5], Elsa Quintana[5], A. Cole Edwards[2], Connor J. Hennessey[2], Austin L. Good[2], Liz Quinones[2], Yunseo Hwang[3], Cynthia Clendenin[2], Ashley L. Kiemen[3,6,7,8], Robert H. Vonderheide[1,2]*, Ben Z. Stanger[1,2]*

Transformation of pancreatic epithelial cells to malignant pancreatic ductal adenocarcinoma (PDAC) typically involves the progression of precancerous pancreatic intraepithelial neoplasia (PanINs) bearing oncogenic *KRAS* mutations. Here, we tested the impact of PDAC interception using either RAS(ON) multiselective or RAS(ON) G12D-selective pharmacological inhibitors [RAS(ON) inhibitors] in mouse models of PDAC. Treatment of PanIN-bearing mice with RAS(ON) inhibitors prompted regression of premalignant lesions that translated into a delay in tumor onset and an increase in overall survival (OS). Long-term interception in tumor-prone mice resulted in a median OS of more than 1 year compared with less than 5 months in nonintercepted control mice ($P < 0.0001$). Comparing the survival benefits of RAS(ON) inhibition for cancer interception versus RAS(ON) inhibition for cancer treatment, we found that interception provided a greater survival benefit to mice. These findings suggest that a pharmacological approach may reduce premalignant burden and increase survival in PDAC.

An alternative to the long-standing paradigm of treating cancer after diagnosis is "cancer interception," a strategy of eliminating non-invasive neoplastic lesions before invasion and metastasis (*1*, *2*). Removal of adenomatous polyps by polypectomy and destruction of dysplastic skin neoplasias by cryotherapy are examples of "mechanical" interception that can be repeated every several years in at-risk individuals. However, mechanical interception is only practical in easy-to-access tissues with identifiable and anatomically defined precursors.

Pancreatic ductal adenocarcinoma (PDAC) currently carries a 5-year survival of <13%, and its incidence is increasing (*3*). Despite substantial efforts, the early detection of PDAC remains elusive, and most patients are diagnosed at a late, inoperable stage that is difficult to treat meaningfully. Most PDAC tumors arise from microscopic neoplastic lesions known as pancreatic intraepithelial neoplasia (PanINs). It is now well known that low-grade PanIN lesions are ubiquitous in healthy adults (*4*, *5*), and >92% of these lesions carry mutations in the *KRAS* oncogene (*6*). By contrast, high-grade PanINs are characterized by tumor suppressor gene alterations, mirroring the genetic signatures of invasive PDAC (*4*, *6*, *7*). These neoplastic precursors are well modeled in autochthonous KPC mice, which are genetically engineered to carry $Kras^{G12D}$ and $Tp53^{R172H}$ mutations throughout the pancreatic epithelium and progress from acinar-to-ductal metaplasia (ADM) to PanIN to invasive PDAC with predictable kinetics (*8*).

Recent advances in pharmacology have led to an array of small-molecule inhibitors of the KRAS oncoprotein that have rapidly advanced from preclinical to clinical development due in part to their safety profile (*9*–*11*). Major clinical activity, including tumor regressions and prolonged survival, have been reported and have impressively paralleled the antitumor activity observed in multiple mouse models including the KPC model (*9*, *12*). Because almost all PanIN lesions (and PDAC tumors) harbor *KRAS* mutations, we hypothesized that inhibiting KRAS early in neoplastic progression might reduce PanIN burden to intercept PDAC. Here, we report that inhibiting KRAS at the premalignant stage has an unprecedented short- and long-term impact, providing a rationale for pharmacological interception of PDAC in high-risk patients.

Results

RAS(ON) inhibition decreases preneoplastic burden

We began our studies by characterizing the timing of PanIN-to-PDAC progression in KPC animals. KPC mice developed palpable PDAC at a median age of 19.1 ± 5.3 weeks ($n = 997$) with nearly 100% penetrance by 38 weeks; overt tumor development before 9 weeks of age was uncommon. By 6 weeks of age, all mice had detectable preneoplasia (PanINs and closely associated ADMs), and we observed an increase in the number and size of these lesions between 7 and 12 weeks of age (fig. S1, A and B). On the basis of these findings, we started interception in animals 9 weeks or younger after confirming the absence of tumor by high-resolution ultrasound (Fig. 1A).

To determine the effects of inhibitors of the active, guanosine triphosphate (GTP)–bound state of RAS isoforms [i.e., RAS(ON) inhibition] on preneoplastic burden, we treated 9-week-old KPC animals with 10 daily doses of either RMC-7977, a RAS(ON) multiselective inhibitor with activity against all RAS proteins including wild-type RAS (*13*, *14*), or RMC-9945, a RAS(ON) G12D-selective inhibitor (*15*, *16*). Quantification of neoplasia revealed a significant decrease after treatment with either inhibitor (Fig. 1, B and C). We confirmed this effect in three-dimensional (3D) space using CODA, a technique for cubic-centimeter-scale reconstruction and anatomical quantification from serial histological sections (*4*, *17*). In mice, CODA can reconstruct entire organs, enabling exhaustive screening of the exocrine pancreas for determination of the number and size of PanINs across experimental conditions (Fig. 1D; figs. S1C and S2, A to D; and movies S1 to S4). Thus, short-term administration of either RMC-7977 or RMC-9945 leads to a significant reduction in pancreatic preneoplasia in KPC mice.

Possible explanations for preneoplastic regression include cell death or changes in cellular differentiation (e.g., acinar redifferentiation), as previously reported (*18*). Because lesions were rare after 10 daily doses of RMC-7977 and RMC-9945, we examined pancreata after three doses, a time point when PanINs were still abundant, for evidence of either phenomenon. As expected, staining for phosphorylated extracellular signal–regulated kinase (pERK), a downstream target of RAS signaling, was markedly diminished by RAS(ON) inhibition (Fig. 1E, top panels). Residual lesions exhibited increased staining for cleaved caspase 3 (CC3), indicating apoptotic cell death (Fig. 1E, middle panels). By contrast, staining for amylase, an acinar cell marker, was unchanged between control- and RAS(ON) inhibition–treated samples, and minimal amylase staining was detected within the PanIN epithelium (Fig. 1E, bottom panels). These findings indicate that RAS(ON) inhibition promotes PanIN regression by inducing cell death in neoplastic epithelial cells, with acinar redifferentiation playing a minor or negligible role at this time point.

In addition to their epithelial component, PanIN lesions have an associated stromal infiltrate consisting mainly of fibroblasts and macrophages (*19*). We and others have previously reported that RAS(ON)

[1]Department of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [2]Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA. [3]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD, USA. [4]Department of Pathology, University of Pennsylvania, Philadelphia, PA, USA. [5]Revolution Medicines, Redwood City, CA, USA. [6]Sol Goldman Pancreatic Cancer Research Center, Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD, USA. [7]Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD, USA. [8]Department of Functional Anatomy & Evolution, Johns Hopkins School of Medicine, Baltimore, MD, USA. ***Corresponding author. Email: bstanger@upenn.edu (B.Z.S.); rhv@upenn.edu (R.H.V.)**

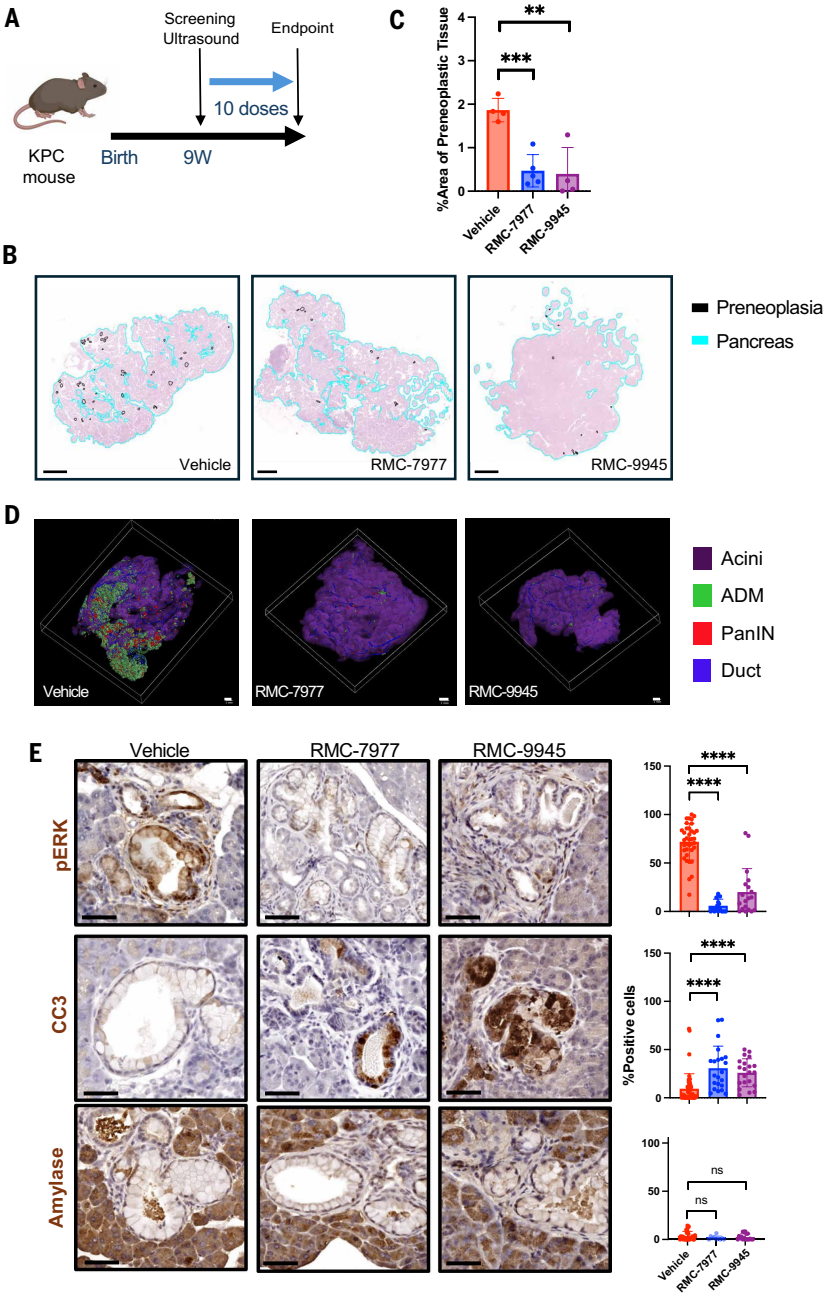

Fig. 1. RAS inhibition decreases PanIN burden. (**A**) Schematic of experimental design. Autochthonous KPC animals (9 weeks old; $n = 3$ to 5) were screened by high-resolution ultrasound to rule out early tumor formation and then treated with vehicle, RMC-7977 (25 mg/kg daily), or RMC-9945 (100 mg/kg daily) by oral gavage for a total of 10 doses before euthanasia. (**B**) Hematoxylin and eosin (H&E) images of pancreas sections from independent mice that received the indicated treatment. Black outlines denote preneoplastic areas. Cyan outlines denote pancreatic tissue. Scale bars, 2 mm. (**C**) Bar graph showing the mean ± SD of the percentage area of preneoplastic tissue under each condition ($n = 4$ to 5 independent animals per group). Each point represents the mean of two pancreas sections (at least 100 μm apart) from an individual animal, with preneoplastic tissue defined as lesions containing ADM and/or PanIN. Statistical significance was determined by unpaired *t* test (**$P < 0.01$, ***$P < 0.001$). (**D**) Representative 3D microanatomy from CODA performed on individual mouse pancreata treated with 10 doses of the indicated compound starting at 9 weeks of age. (**E**) Left, representative IHC images of pERK, CC3, and amylase staining of PanIN lesions in animals that received the indicated treatment for 3 days and were euthanized 4 hours after the last dose. Right, bar graphs showing the percentage of positive cells per PanIN area for each stain. Each dot represents an individual PanIN region, with two to three PanINs evaluated per animal ($n = 3$ to 5 animals). Statistical significance was determined by unpaired *t* test (****$P < 0.0001$). Scale bars, 50 μm.

inhibition in established PDAC tumors reshapes the tumor microenvironment (TME) (*20*–*22*); therefore, we examined the effects of short-term RAS(ON) inhibition on perineoplastic regions, the "pre-TME." Further immunohistochemical (IHC) analysis revealed a small decrease in F4/80$^+$ macrophages associated with the residual lesions after 3 days of RMC-7977 treatment, whereas the number of podoplanin$^+$ fibroblasts and CD3$^+$ T cells remained unchanged relative to controls (fig. S3, A and B). We additionally evaluated an earlier time point, 8 hours after a single dose, which revealed similar changes in F4/80$^+$ macrophages (fig. S4, A and B). We used CODA to further quantify the local inflammation surrounding PanIN lesions in 3D. To this end, we measured immune cell infiltration (cells considered immune by the CODA algorithm) within a 125-μm spherical radius around PanIN lesions remaining after 10 days of treatment. This revealed a decrease in immune infiltration surrounding PanINs treated for 10 days with RMC-7977 (fig. S4C). Thus, RAS(ON) inhibition prompts modest effects on the pre-TME before regression of PanIN lesions, with changes more readily apparent when viewed in 3D space.

To assess the functional contributions of T cells to RAS(ON) inhibition–mediated PanIN regression, we depleted CD4$^+$ and CD8$^+$ T cells in KPC mice treated with RMC-7977 and did not observe differences in preneoplastic burden (fig S4, D and E). Thus, T cells within the pre-TME do not appear to affect the acute regression of preneoplastic lesions upon RAS(ON) inhibition.

RAS(ON) inhibition intercepts PDAC and extends survival

To evaluate the longer-term impact of RAS(ON) inhibition on precancerous lesions, we treated tumor-free, 7-week-old KPC mice for 28 days with vehicle, RMC-7977, or RMC-9945 (Fig. 2A). Evaluation of pancreata after 28 days of treatment with either compound revealed suppression of preneoplasia to an equivalent or greater extent than 10 days of treatment (Fig. 2B and fig. S5A). RMC-9945 fully eliminated detectable preneoplasia in two of five animals. After this 28-day "interception period," we discontinued inhibitor treatment and observed animals for tumor formation and overall survival (OS). As expected, all animals eventually succumbed to PDAC tumors. However, interception with RMC-7977 resulted in a significant delay in tumorigenesis and progression-free survival (PFS) (median age at tumor onset 136 days versus 94 days; $P = 0.0023$) and improved OS (median age at death 159 days versus 135 days; $P = 0.007$) (Fig. 2C). An even greater delay in tumorigenesis was observed with the RAS(ON) G12D-selective inhibitor RMC-9945 (median tumor onset 162 days versus 83 days; $P < 0.0001$), which translated into an ~80-day prolongation of OS (median age at death 200 days versus 120 days; $P < 0.0001$) (Fig. 2D). Although both compounds blunted weight gain over the interception interval, neither compound was associated with prominent weight loss (fig. S5B).

To understand the consequences of premalignant interception on subsequent tumor development, we assessed the growth of tumors that received vehicle or RAS(ON) inhibition in the premalignant setting. Tumors that emerged with delayed kinetics after interception

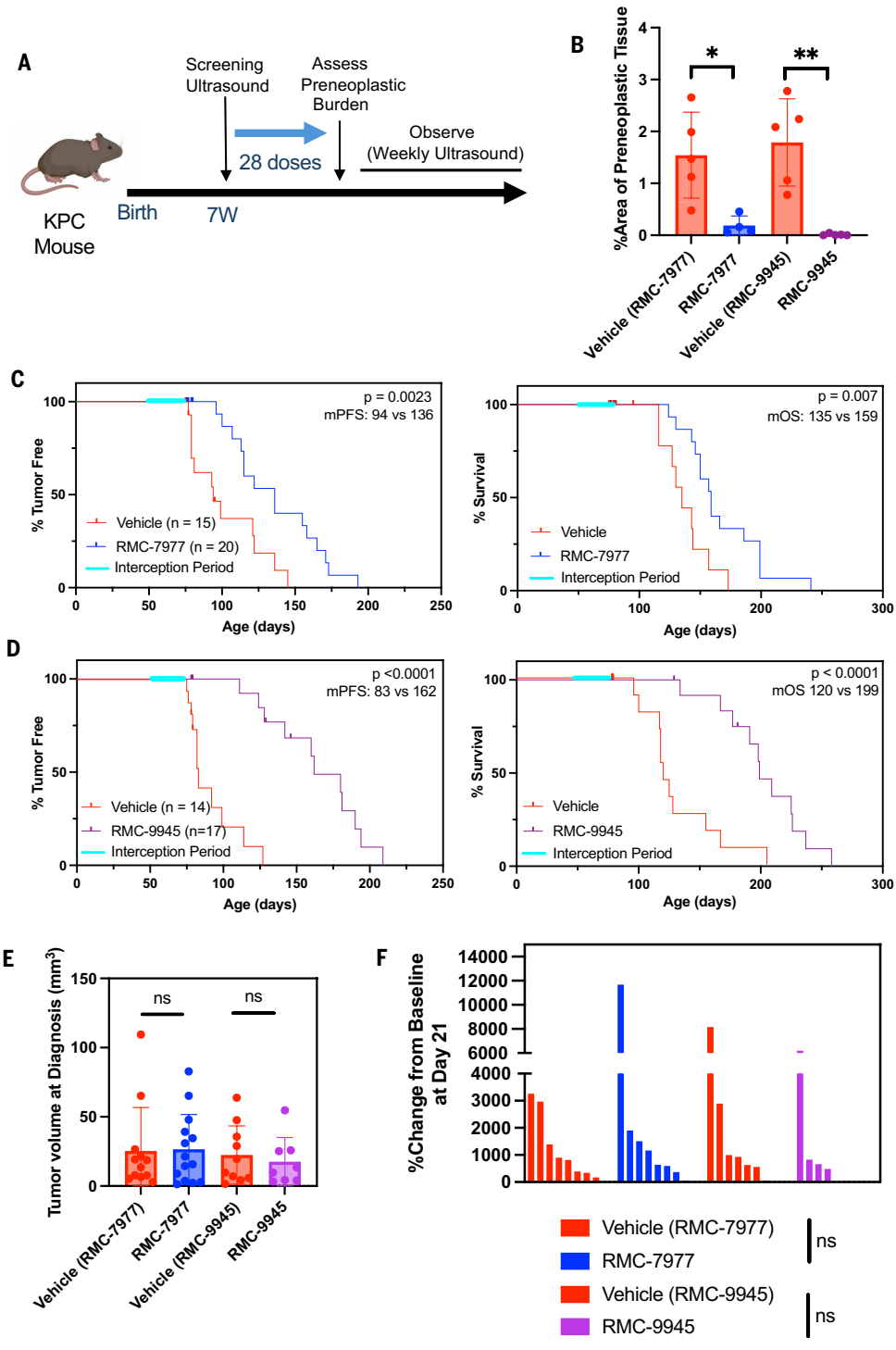

Fig. 2. Induction dose of RAS(ON) inhibition delays tumor onset and prolongs OS. (**A**) Schematic of experimental design. Autochthonous KPC animals (7 weeks old) were screened by high-resolution ultrasound to rule out early tumor formation and then treated with vehicle, RMC-7977 (25 mg/kg daily), or RMC-9945 (100 mg/kg daily) by oral gavage for a total of 28 doses. A subset of mice was euthanized at the 28-day time point for quantification of preneoplasia, whereas others were continually monitored by ultrasound for tumor development. (**B**) Bar graph showing the mean ± SD of the percentage area of preneoplastic tissue for each condition ($n = 4$ to 5 independent animals per group) at the end of the 28-day interception period. Each point represents the mean of two pancreas sections (at least 100 μm apart) from an individual animal, with preneoplastic tissue defined as lesions containing ADM and/or PanIN. Statistical significance was determined using unpaired t test (*$P < 0.05$, **$P < 0.01$). (**C**) Kaplan-Meier curves comparing tumor-free survival (left) and OS (right) of animals treated for 28 days with RMC-7977 and observed. Statistical significance was determined by log-rank test. (**D**) Kaplan-Meier curves comparing tumor-free survival (left) and OS (right) of animals treated for 28 days with RMC-9945 and observed. Statistical significance was determined by log-rank test. (**E**) Bar graph showing mean ± SD of tumor size at the time of diagnosis for each condition ($n = 8$ to 12 independent animals per group). Statistical significance was determined by unpaired t test (ns, not significant). (**F**) Waterfall plot showing change in tumor volume for the 3-week period after diagnosis in tumors arising after interception or vehicle. Statistical significance was determined by Mann-Whitney U test (ns, not significant).

were similar in size and grew at a similar rate to those that arose in the vehicle control group (Fig. 2, E and F, and fig. S5C), suggesting that interception did not select for more aggressive tumors. Accordingly, OS measured from the time of tumor detection was similar among RMC-7977–intercepted animals, RMC-9945–intercepted animals, and their respective controls (fig. S5, D and E). Thus, short-term RAS(ON) inhibition with multiselective and allele-specific inhibitors delays tumor onset and prolongs survival to an extent that matches or exceeds the duration of interception without selecting for more lethal tumors.

In the autochthonous KPC mouse model, mutations in *Kras* and *Tp53* are present throughout the pancreatic epithelium; therefore, new PanINs arise continuously and with faster kinetics than they do in the human population. This aspect of the genetic model likely explains our observation that a 28-day interception period delays tumor onset by only a comparable interval (30 to 60 days). To test for an enduring effect on survival with long-term use of RAS(ON) inhibition, we devised a metronomic delivery schedule in which we screened 7-week-old KPC mice for absence of tumors and then assigned them to receive either RMC-7977 ($n = 17$) or vehicle control ($n = 12$) on a

1 week ON/1 week OFF dosing routine (Fig. 3A). A metronomic delivery schedule was favored to provide low-dose exposure over a longer period of time, allowing us to limit theoretical toxicity in a manner similar to other cancer-directed pharmacologic interventions (*23*–*25*). All but one animal in the control group developed lethal tumors within 150 days of enrollment, with the final mouse being euthanized for development of a facial tumor (Fig. 3, B and C). By contrast, RMC-7977–intercepted animals had markedly delayed tumor onset and tripling of median tumor-free survival (median age at tumor onset 329 days versus 105 days; $P < 0.0001$) (Fig. 3, B and C), with a portion of animals dying instead from non–PDAC-related causes such as soft tissue sarcomas, likely related to *Tp53* haploinsufficiency (*26*), or rectal prolapse. The delay in tumor development translated into a significant increase in PDAC-related OS (Fig. 3D), with a median OS of 376 days in intercepted mice compared with 138 days in the vehicle control group ($P < 0.0001$). Mice in the RMC-7977 interception group gained weight to a similar extent as vehicle controls over the course of the study (≥6 months) (fig. S6A).

RAS(ON) inhibitor treatment of tumors, dosed from the time of diagnosis, provides significant antitumor benefit in both implantable and KPC models of PDAC (*13*, *14*, *27*). To compare the relative benefit of interception with RAS(ON) inhibition with the benefit of initiating RAS(ON) inhibition at diagnosis, we treated a separate cohort of tumor-bearing mice with the same metronomic delivery schedule (1 week ON/1 week OFF). By administering the same metronomic delivery schedule but initiating at different points during tumorigenesis, we aimed to delineate the benefit of earlier intervention and interception. Although metronomic dosing with RAS(ON) inhibition improved survival in tumor-bearing animals, interception provided a superior survival benefit (fig. S6, B and C). We further compared the survival of our interception with two historic KPC cohorts and found that interception provided an unprecedented prolongation of survival (fig. S6D). Nevertheless, RAS(ON) inhibition interception did not restore a normal lifespan in the KPC model, because survival was still significantly reduced compared with *Tp53*-haploinsufficient animals lacking *Kras* mutations (PC mice) (fig. S6E).

To assess whether preneoplasia remained suppressed with long-term metronomic RAS(ON) inhibition, we measured the preneoplastic area in RMC-7977–intercepted animals and compared it with the same area in vehicle-treated controls (excluding tumor regions). Whereas vehicle-treated animals exhibited substantial preneoplasia ($7.66 \pm 2.23\%$), RMC-7977–intercepted animals had a preneoplastic burden ($0.64 \pm 0.25\%$) that was comparable to that observed in younger mice with short-term exposure to RMC-7977 ($0.47 \pm 0.37\%$) (Fig. 3E and fig. S7A). To assess whether long-term metronomic treatment results in changes in the pancreatic microstructure, we then applied CODA to three long-term survivors from the RMC-7977 interception study. This revealed a similar composition of vasculature and acinar cells and a small increase in islet volume compared with younger mice treated for 10 days with vehicle (fig. S7B). Thus, extended interception with RMC-7977, delivered in metronomic fashion, results in the durable suppression of PanINs and ADMs and a significant survival benefit without disrupting pancreatic architecture.

Interception does not select for more aggressive tumor phenotypes

The KPC tumors that arose in the RMC-7977 interception group were comparable in size at diagnosis and exhibited similar histology to those emerging in vehicle-treated controls (Fig. 3F and fig. S8, A and B). To determine whether these tumors that "escaped" interception behaved differently from tumors that arose in the absence of RAS(ON) inhibition, we divided the interception cohort into two groups at the time of tumor detection: an "interception and treat" group, which continued to receive RMC-7977 on a 1 week ON/1 week OFF schedule, and an "interception and watch" group, which received no further intervention (Fig. 3A). A few tumors in the "interception and treat" group exhibited delayed tumor growth compared with tumors that received no further treatment (fig. S8C), indicating that tumors that emerge despite interception may remain partially responsive to RAS(ON) inhibition. Indeed, tumors in the "interception and treat" group exhibited similar growth kinetics to those that arose in the absence of interception treated with the same metronomic regimen (metronomic RMC-7977 at diagnosis) KPC tumors (fig. S8D). Accordingly, animals in the "interception and treat" group exhibited increased OS compared with vehicle-treated animals (median survival from diagnosis 29 days versus 56 days; $P = 0.0116$) and a trend toward better survival compared with the "interception and watch" group (39 days versus 56 days; $P = 0.2354$) (Fig. 3G). Thus, tumors that "escape" interception seem no more lethal than their spontaneously arising counterparts and may retain sensitivity to RAS inhibition despite emerging in the setting of interception with a RAS(ON) inhibitor.

To explore whether escape from interception might have a genetic basis, we propagated vehicle-treated (nonintercepted) tumors and "interception and watch" (intercepted) tumors as 2D cell lines. We performed whole-genome sequencing and and assessed whether copy number changes in members of the mitogen-activated protein kinase family (*Mapk*), *Myc*, or *cyclophilin A* (*Cypa*) were more prevalent in intercepted tumors (fig. S9A). Copy number gains in *Myc* were the same in both groups (four of five). Copy number gains in KRAS and BRAF were each found in a single tumor in each group. There was one tumor in the intercepted group that had an amplification in *Map2k1* (*Mek1*). There were no copy number losses in *Cypa* in either group, and manual assessment of *Kras* and *Cypa* sequences failed to reveal mutations that could explain resistance to RAS(ON) inhibition. Likewise, reproducible differences in copy number were not observed at other loci. Finally, we implanted the propagated cell lines subcutaneously into immunocompetent mice and exposed the resulting tumors to daily treatment with RMC-7977. Subcutaneous tumors derived from both nonintercepted and intercepted cell lines had delayed growth kinetics when treated with daily RMC-7977 (fig. S9B), indicating that they remained responsive to RAS inhibition. Together, these data suggest that the mechanism of escape in this small sample is not genetically enforced.

Discussion

Cancer prevention approaches such as smoking cessation and HPV vaccination have substantially reduced the risk of their associated cancers (*28*, *29*). Similarly, mechanical or procedure-based cancer interception approaches such as colonoscopy with polypectomy, which eliminates colorectal adenomas before invasion and metastasis, have led to a substantial reduction in colorectal cancer incidence and mortality (*30*). But for PDAC, which is predicted to become the second-leading cause of cancer death in the US within 5 years, effective and broadly applicable approaches to early detection, prevention, and interception are lacking. Our data show that interception can be accomplished by targeting the nearly universal driving oncogene in this disease, mutant KRAS, and effectively forestalling tumor development even in a highly aggressive model of premalignant-to-malignant progression.

Here, RAS(ON) inhibition with RMC-7977 or RMC-9945 led to a reduction in preneoplastic burden after only 10 days and was associated with inhibition of MAPK signaling and induction of cell death by apoptosis instead of promoting acinar redifferentiation. Although interception led to minimal changes in fibroblasts and T cells, there was a decrease in $F4/80^+$ macrophages and possibly other immune cells in the setting of RMC-7977 interception. These findings are consistent with prior findings demonstrating macrophage sensitivity to this compound (*31*). Notably, we failed to observe clusters of residual fibroblasts and/or macrophages in non-PanIN regions. This suggests that KRAS signaling is critical for pre-TME maintenance and that these cell populations regress rapidly when KRAS is inhibited. Pre-TME changes may be more readily detected with orthogonal methods such

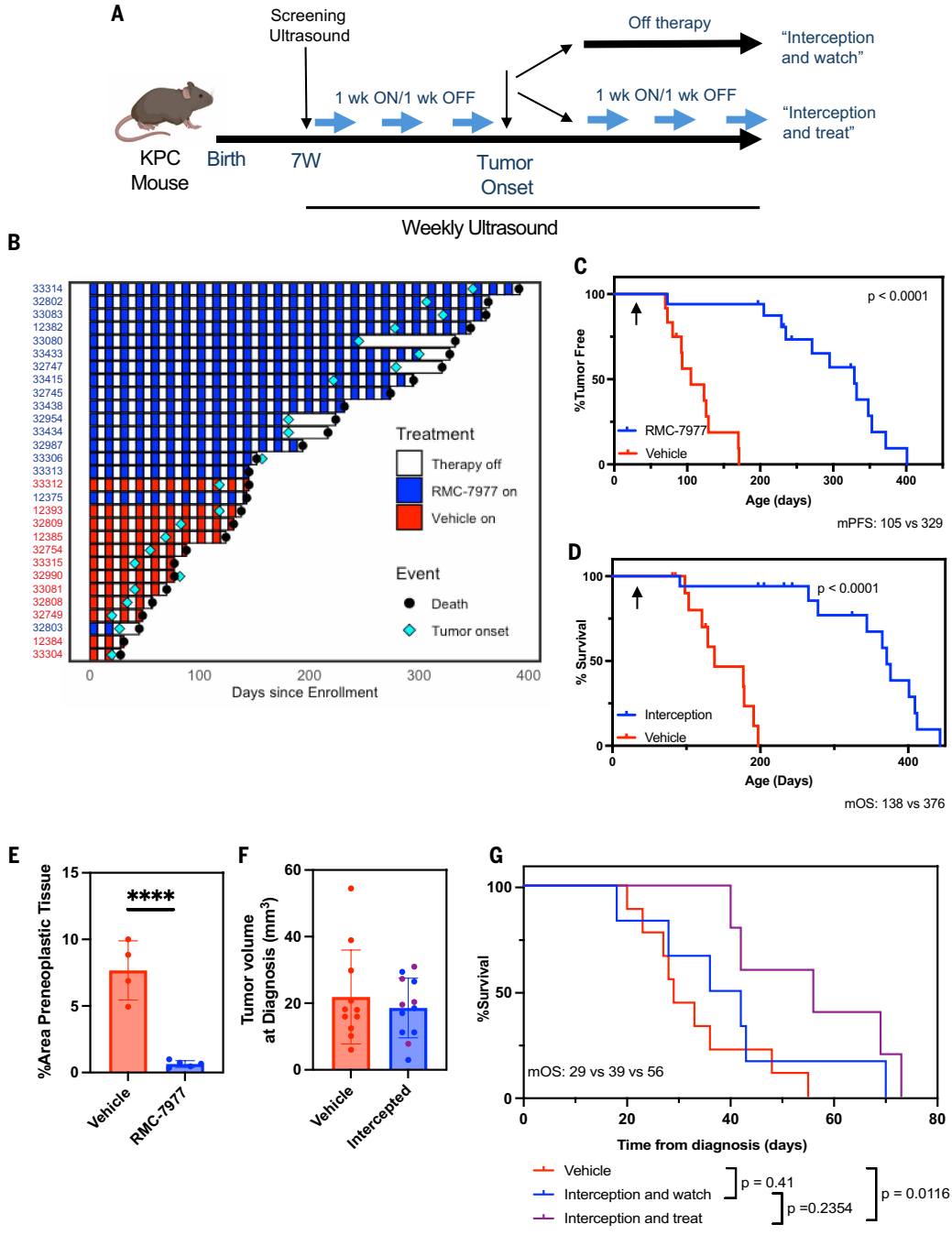

Fig. 3. Long-term RAS(ON) inhibition delays tumor onset and improves OS. (**A**) Schematic of experimental design for the long-term interception experiment. Tumor-free, 7-week-old KPC animals were given either RMC-7977 (25 mg/kg daily) or vehicle control for alternating weeks (7 day ON treatment/7 day OFF treatment) and observed by weekly ultrasound until tumors developed and/or mice became moribund. Mice in the RMC-7977 treatment cohort were separated into two groups at the time of tumor detection: an "interception and watch" group (no therapy) and an "interception and treat" group (continued to receive RMC-7977 on a 7 day ON/7 day OFF schedule). (**B**) Swimmers plot depicting time on study (starting at 7 weeks of age), treatment received (white is no therapy, red is vehicle, and blue is RMC-7977), tumor onset (cyan diamond), and animal death due to any cause (black circle). Each row represents an individual animal. See fig. S4A for a detailed schematic of the study design. (**C**) Kaplan-Meier plot depicting percentage of tumor-free survival for each experimental arm. The arrow indicates the initiation of interception. Median progression-free survival (mPFS) is shown in days. Statistical significance was determined by log-rank test. (**D**) Kaplan-Meier plot depicting the percentage OS for each experimental arm. The arrow indicates the initiation of interception. All curves reflect PDAC-specific survival; animals euthanized for non–PDAC-related reasons were censored in this analysis. Median OS (mOS) is shown in days. Statistical significance was determined by log-rank test. (**E**) Bar graph showing the mean ± SD of the percentage of preneoplastic tissue in nontumor regions of animals that received RMC-7977 or vehicle control ($n = 4$ to 5 individual animals per group). Each point represents the mean of two pancreas sections (at least 100 μm apart) from an individual animal, with preneoplastic tissue defined as lesions containing ADM and/or PanIN. RMC-7977–intercepted animals assessed were all tumor free at a median age of 232 days. Vehicle-treated animals were all tumor bearing at a median age of 173 days, with quantification limited to non-tumor-involved areas. Statistical significance was determined by unpaired *t* test (****$P < 0.0001$). (**F**) Bar graph showing the mean ± SD of tumor size at diagnosis ($n = 10$ to 12 independent animals per group). Each point represents a single animal. Blue dots represent "interception and watch" tumors, and purple dots represent "interception and treat" tumors. Error bars indicate SD. Statistical significance was determined by unpaired *t* test. (**G**) Kaplan-Meier curves depicting survival from time of tumor diagnosis under each condition. Statistical significance was determined by log-rank test.

as spatial technologies. Although we observed comparable effects with RMC-7977 [a RAS(ON) multiselective inhibitor] and RMC-9945 (a $KRAS^{G12D}$-selective inhibitor), there were some differences. RMC-9945 treatment resulted in greater preneoplastic suppression after 28 days of treatment than did RMC-7977, with an almost complete elimination of PanIN and ADM. This differential effect may explain the differences in subsequent tumor onset with each agent. Nevertheless, our data indicate that interception does not require complete elimination of neoplastic lesions and that even some reduction in preneoplastic burden can affect subsequent tumorigenesis.

Extended interception using a 1 week ON/1 week OFF metronomic RMC-7977 dosing schedule led to a tripling of animal survival, even more noteworthy because all pancreatic epithelial cells in the KPC model harbor cancer-causing mutations and generate new neoplastic lesions on an ongoing basis and with high frequency. The prolonged benefit of intervening on PanIN compared with fully formed invasive cancer is consistent with the well-articulated concept that early disease is less complex and therefore less adaptable (*32*). Therefore, we speculate that PanIN lesions lack the genomic instability or molecular plasticity to readily compensate for loss of KRAS signaling. At least some of the tumors that emerge despite long-term interception with RMC-7977 benefit from ongoing treatment with the inhibitor, suggesting that such tumors remain dependent on KRAS-MAPK signaling for growth. The extent of benefit of interception and the sensitivity of tumors after interception highlights ongoing "oncogene addiction" of PDAC to KRAS-MAPK signaling. Increasing the frequency of RAS(ON) inhibition upon tumor diagnosis (from metronomic to daily dosing) may contribute further to this benefit. Our data suggest that the mechanisms of escape from interception may be nongenetic and rely instead on cellular plasticity or microenvironmental factors. Alternatively, given that "interception and watch" tumors were evaluated in our study, it is possible that the off-therapy "watch" period allowed inhibitor-sensitive, KRAS-dependent cells to outcompete inhibitor-resistant cells.

PanINs are ubiquitous in the adult population but are only detectable microscopically. Therefore, clinical translation of interception, at least given current technology, would likely use clinical risk as a basis for eligibility. This would include individuals with a genetic predisposition for PDAC, such as carriers of *BRCA1*, *BRCA2*, and *PALB2* mutations and those with hereditary pancreatitis or other strong risk factors for which age-dependent risk is increasingly understood (*33*, *34*). In this regard, knowing the predetermined risk of cancer development may obviate the need to preemptively identify these microscopic lesions patient by patient. We have recently outlined a roadmap for cancer interception (*2*) that may include immunological and vaccine intervention that could be combined with the targeted strategy described here. A major strength of the interception approach is the ability to eliminate lesions before genomic instability and other mechanisms of resistance have time to mature. Nevertheless, because these approaches would involve individuals without a cancer diagnosis, it will be critically important to establish safety and tolerability.

Several RAS inhibitors are under development for the treatment of patients with RAS-mutant cancers, including daraxonrasib, a close chemical relative of RMC-7977 (*9*, *10*), and zoldonrasib (*15*, *16*), a close chemical relative of RMC-9945. Given the multifocality of PanIN lesions and the spectrum of KRAS mutations seen in human PanINs, RAS(ON) multiselective inhibitors may have more utility than allele-specific inhibitors in the setting of interception. Our study suggests that as the safety and efficacy of these agents are established in invasive PDAC, it may be feasible and appropriate to bring them forward to the premalignant setting.

REFERENCES AND NOTES

1. E. H. Blackburn, *Cancer Prev. Res.* **4**, 787–792 (2011).
2. S. M. Domchek, R. H. Vonderheide, *Cancer Discov.* **14**, 600–604 (2024).
3. American Cancer Society, "Cancer facts & figures 2025" (2025); https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2025-cancer-facts-figures.html.
4. A. M. Braxton *et al.*, *Nature* **629**, 679–687 (2024).
5. E. S. Carpenter *et al.*, *Cancer Discov.* **13**, 1324–1345 (2023).
6. M. Kanda *et al.*, *Gastroenterology* **142**, 730–733.e9 (2012).
7. N. Waddell *et al.*, *Nature* **518**, 495–501 (2015).
8. S. R. Hingorani *et al.*, *Cancer Cell* **7**, 469–483 (2005).
9. J. Jiang *et al.*, *Cancer Discov.* **14**, 994–1017 (2024).
10. J. Cregg *et al.*, *J. Med. Chem.* **68**, 6064–6083 (2025).
11. T. Isermann, C. Sers, C. J. Der, B. Papke, *Trends Cancer* **11**, 91–116 (2025).
12. K. C. Arbour *et al.*, *Ann. Oncol.* **34**, S458 (2023).
13. M. Holderfield *et al.*, *Nature* **629**, 919–926 (2024).
14. U. N. Wasko *et al.*, *Nature* **629**, 927–936 (2024).
15. N. Tovbis Shifrin *et al.*, *J. Immunother. Cancer* **11**, A242 (2023).
16. C. Weller *et al.*, *Science* **389**, eads0239 (2025).
17. A. L. Kiemen *et al.*, *Nat. Methods* **19**, 1490–1499 (2022).
18. M. A. Collins *et al.*, *J. Clin. Invest.* **122**, 639–653 (2012).
19. A. Velez-Delgado *et al.*, *Cell. Mol. Gastroenterol. Hepatol.* **13**, 1673–1699 (2022).
20. S. B. Kemp *et al.*, *Cancer Discov.* **13**, 298–311 (2023).
21. K. K. Mahadevan *et al.*, *Cancer Cell* **41**, 1606–1620.e8 (2023).
22. M. Orlen *et al.*, *Cancer Discov.* **15**, 1697–1716 (2025).
23. E. Bryson *et al.*, *Clin. Colorectal Cancer* **20**, 153–160 (2021).
24. R. J. Mayer *et al.*, *N. Engl. J. Med.* **372**, 1909–1919 (2015).
25. G. N. Hortobagyi *et al.*, *N. Engl. J. Med.* **375**, 1738–1748 (2016).
26. K. P. Olive *et al.*, *Cell* **119**, 847–860 (2004).
27. M. I. Orlen *et al.*, *Cancer Discov.* **15**, 1697–1716 (2025).
28. H. A. Tindle *et al.*, *J. Natl. Cancer Inst.* **110**, 1201–1207 (2018).
29. P. Dorali *et al.*, *JAMA* **333**, 165–166 (2025).
30. C. A. Doubeni *et al.*, *Gut* **67**, 291–298 (2018).
31. H. A. Araujo *et al.*, *Cancer Discov.* **14**, 2183–2208 (2024).
32. W. N. Hait, P. F. Lebowitz, *Cancer Prev. Res.* **9**, 635–637 (2016).
33. C. Hu *et al.*, *JAMA* **319**, 2401–2409 (2018).
34. B. W. Katona *et al.*, *Cancer* **131**, e35666 (2025).

ACKNOWLEDGMENTS

We gratefully acknowledge Conquer Cancer - Ewa Maria Kukrika Young Investigator Award in Pancreatic Cancer, supported by Miodrag Kukrika, MD. Any opinions, findings, and conclusions expressed in this material are those of the author(s) and do not necessarily reflect those of the American Society of Clinical Oncology® or Conquer Cancer®, or Miodrag Kukrika, MD. We are grateful for the support of the Penn Pancreatic Cancer Research Center and A Love for Life. We thank L. Wood, members of the Stanger and Vonderheide labs, and Revolution Medicines scientists for their advice and suggestions. **Funding:** This work was supported by the National Institutes of Health (grants R01CA276512 and R01CA252225 to B.Z.S. and grant T32 CA009615 to M.T.T.); the Department of the Army (grant W81XWH2210730 to B.Z.S.); the Basser Center for BRCA (R.H.V. and B.Z.S.); Revolution Medicines (sponsored research award to R.H.V. and B.Z.S.); Conquer Cancer (young investigator award to M.T.T.); the Lustgarten Foundation-AACR (career development award to A.L.K.); the Carl and Carol Nale Fund for Pancreatic Cancer Research (A.L.K.); the Rolfe Pancreatic Cancer Foundation (A.L.K.); and the Sol Goldman Pancreatic Cancer Research Center (A.L.K.). **Author contributions:** Conceptualization: M.T.T., R.H.V., B.Z.S.; Funding acquisition: R.H.V., B.Z.S.; Investigation: M.T.T., L.D., R.S., S.N., N.M., E.E.F., C.R.-F., C.C., A.C.E., A.L.G., L.Q., C.J.H.; Methodology: M.T.T., L.D., A.L.K., R.H.V., B.Z.S.; Supervision: A.L.K., R.H.V., B.Z.S.; Visualization: M.T.T., L.D.; Writing – original draft: M.T.T.; Writing – review & editing: M.T.T., R.H.V., B.Z.S., M.M., E.Q. **Competing interests:** R.H.V. has received consulting fees from BMS, Grey Wolf, and EMD Serono and research funding from Revolution Medicines; is an inventor on patents relating to cancer cellular immunotherapy, cancer vaccines, and KRAS immune epitopes; and receives royalties from Children's Hospital Boston for a licensed research-only monoclonal antibody. B.Z.S. receives research funding from Boehringer-Ingelheim and Revolution Medicines. B.Z.S., R.V., and M.T are inventors on a provisional patent application 63/905,781 submitted by the University of Pennsylvania related to KRAS inhibition and cancer interception. The remaining authors declare no competing interests. **Data, code, and materials availability:** All data are available in the main text or the supplementary materials upon request to the corresponding authors. RMC-7977 and RMC-9945 were provided by Revolution Medicines, Inc. (RevMed) under a sponsored research agreement. Any Revolution Medicines, Inc. materials used in the study can be made available directly from the company through a material transfer agreement.

SUPPLEMENTARY MATERIALS

science.org/doi/10.1126/science.aec7929
Materials and Methods; Figs. S1 to S9; References (*35*, *36*); MDAR Reproducibility Checklist; Movies S1 to S4

Submitted 5 October 2025; accepted 15 January 2026

10.1126/science.aec7929

Cancer interception with KRAS inhibitors in preclinical models of pancreatic ductal adenocarcinoma

Minh T. Than, Lucie Dequiedt, Rina Sor, Shreya Nair, Nune Markosyan, Emma E. Furth, Chenghua Yang, Courtney Ray-Fofana, Marie Menard, Elsa Quintana, A. Cole Edwards, Connor J. Hennessey, Austin L. Good, Liz Quinones, Yunseo Hwang, Cynthia Clendenin, Ashley L. Kiemen, Robert H. Vonderheide, and Ben Z. Stanger

Science **391** (6790), . DOI: 10.1126/science.aec7929

Editor's summary

Pancreatic ductal adenocarcinoma (PDAC) is the most common form of pancreatic cancer and is frequently driven by mutations in the *KRAS* gene. Pancreatic intraepithelial neoplasias are precancerous precursor lesions of PDAC that typically harbor the *KRAS* activating mutation. Than *et al*. tested the effects of small-molecule KRAS inhibitor drugs as a chemopreventative strategy for pancreatic cancer (see the Perspective by Neel and Maitra). Using a well-studied model of pancreatic cancer, mice with pancreatic intraepithelial neoplasia lesions were treated with KRAS inhibitors before tumor formation. A series of different dosing regimens were tested, and early intervention was found to extend survival. Three-dimensional mapping of the tumor microenvironment suggested no major distortions in tissue architecture after treatment. The design of clinical trials to determine whether similar effects are observed in humans will be important next steps. —Priscilla N. Kelly