

TAP Off-topic Candidacy: RCE1 inhibition for the treatment of Ras mutated pancreatic ductal adenocarcinoma

NAME OF STUDENT

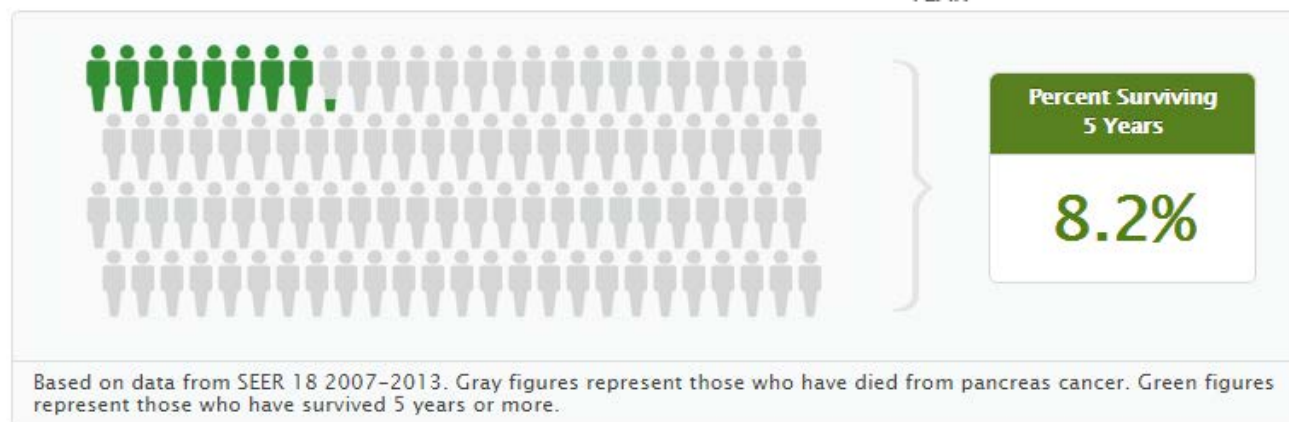
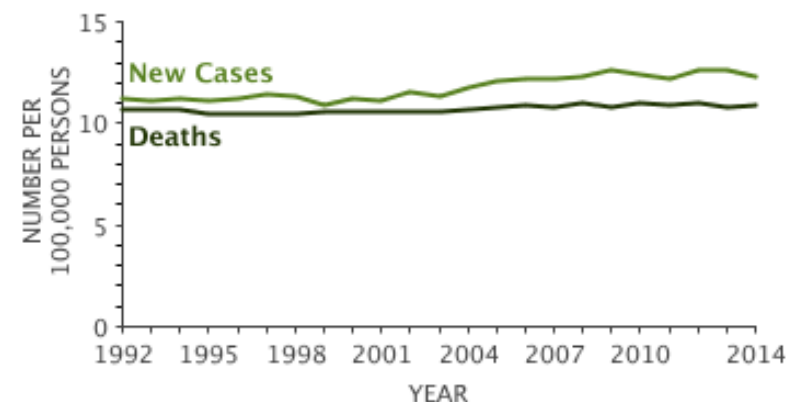
DATE OF EXAM

Pancreatic ductal adenocarcinoma (PDAC) survival statistics illustrate the need for new therapies.

2

- ▶ 5-year survival ~ 8%
- ▶ 4th in US cancer-related deaths
- ▶ Gemcitabine:
 - one-year survival rate ~ 18%
- ▶ PDAC > 85% of pancreatic cancers.

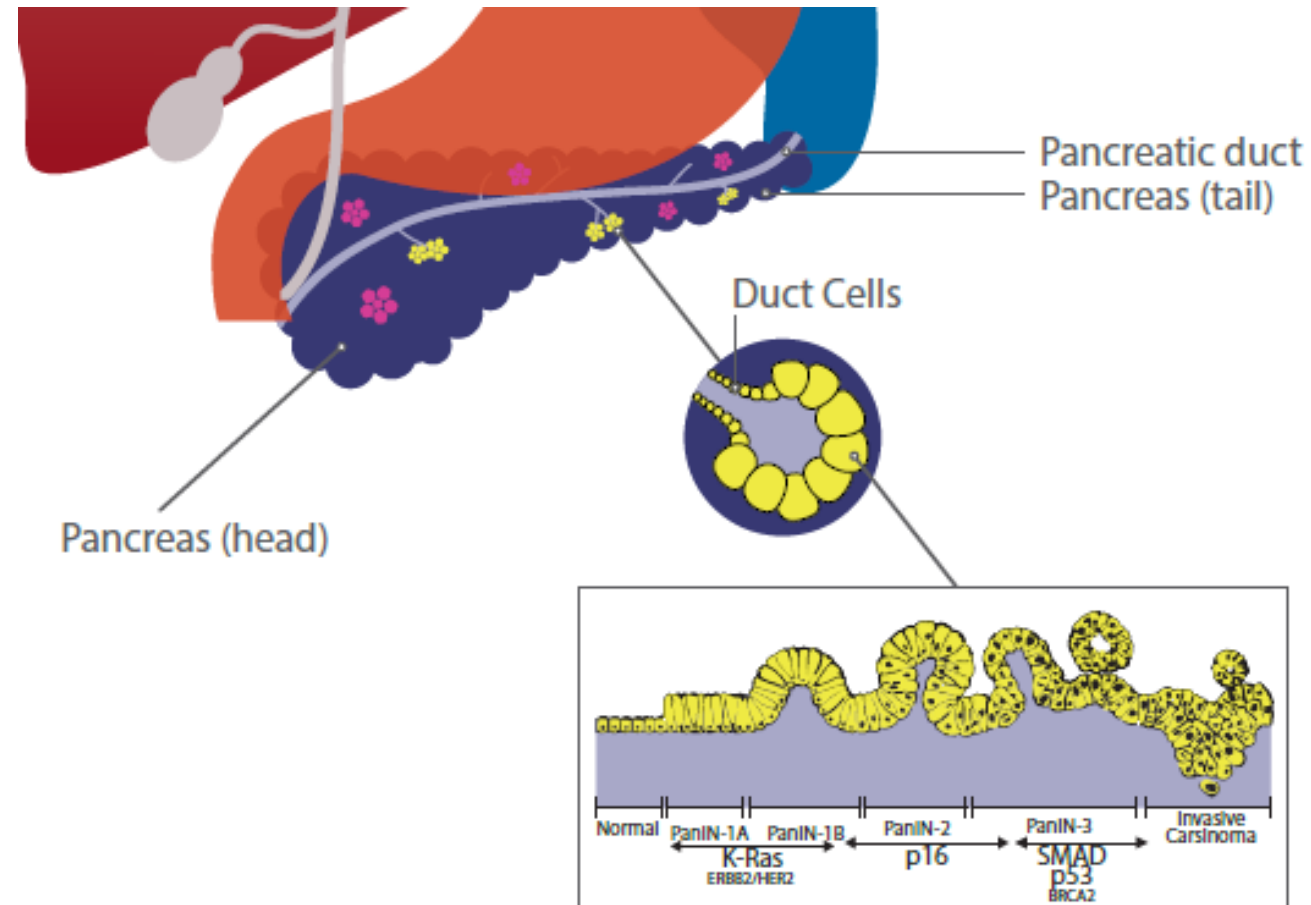
Estimated New Cases in 2017	53,670
% of All New Cancer Cases	3.2%
Estimated Deaths in 2017	43,090
% of All Cancer Deaths	7.2%



Oncogene dependence in PDAC is a rationale for the use of Ras regulation for treatment

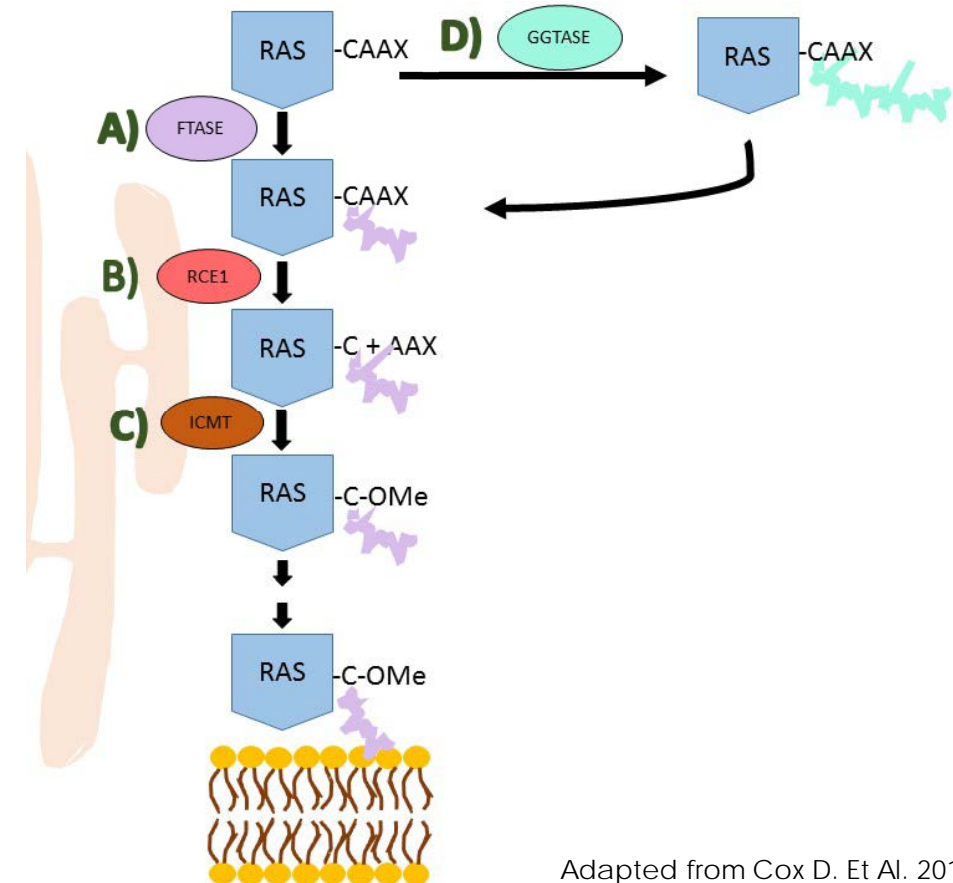
3

- ▶ pancreatic intraepithelial neoplasia (PanINs)
- ▶ Activating mutations of K-Ras > 90% of PDAC patients
- ▶ K-Ras mutations are early genetic events.

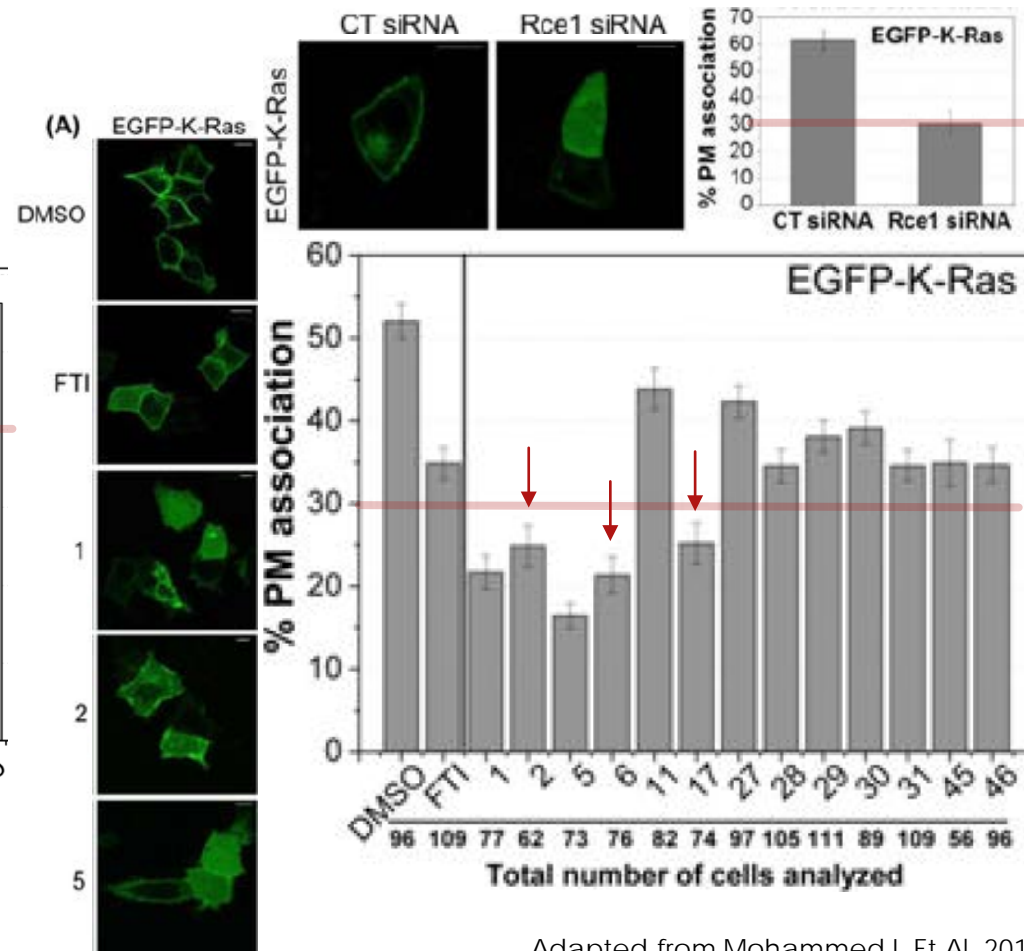
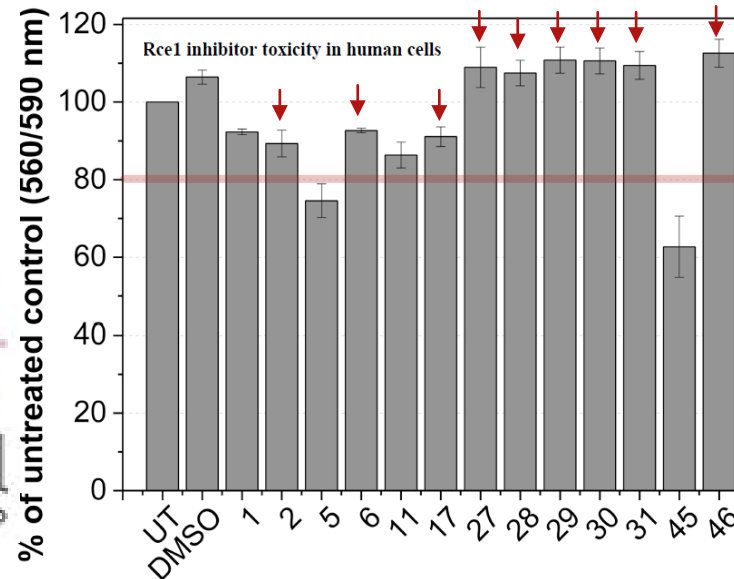
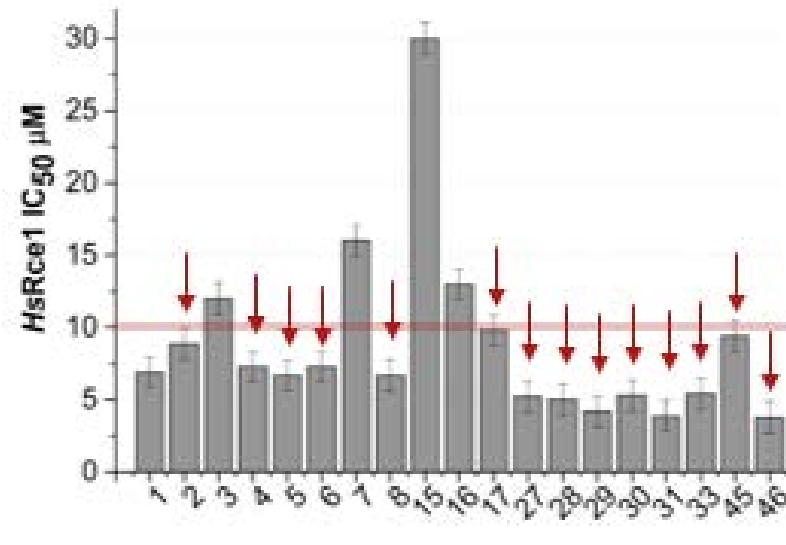


Inhibition of the transport to the cell membrane impedes Ras activity

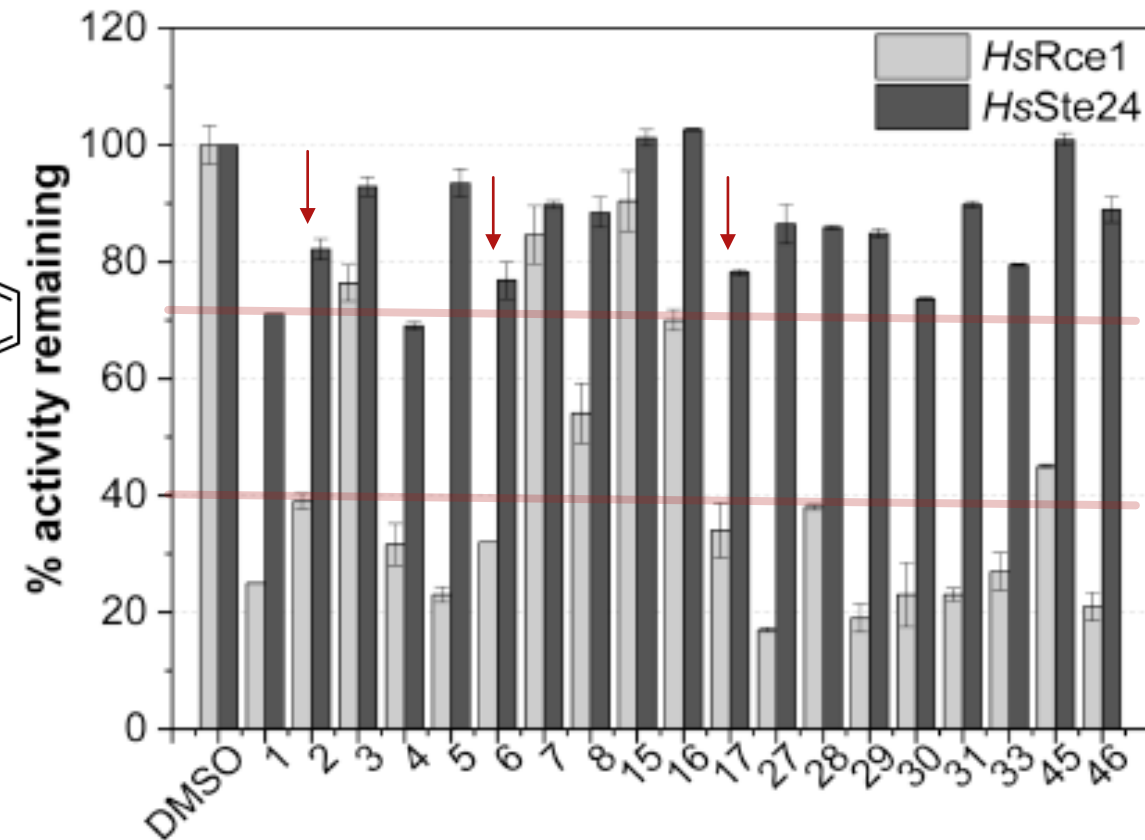
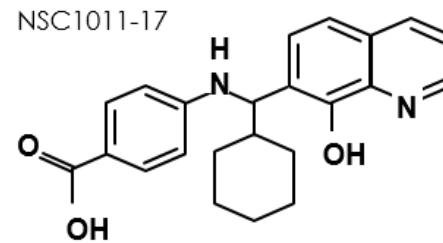
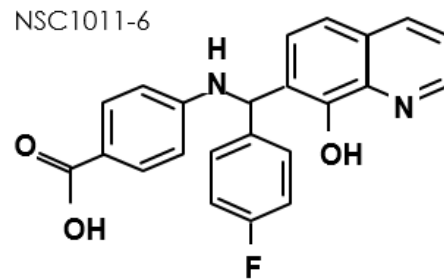
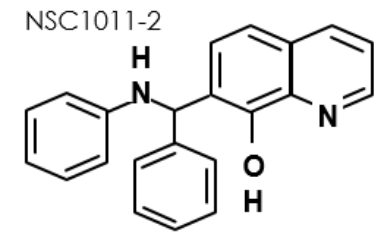
- ▶ (A) Prenylation by farnesyltransferase
- ▶ (B) Cleavage the terminal -AAX motif by Ras converting enzyme 1 (RCE1)
- ▶ (C) Methylation by isoprenylcystein carboxyl methyltransferase (ICMT)
- ▶ (D) Alternative prenylation by geranylgeranyl transferase (GGTASE).



Ras converting enzyme 1 (RCE1) inhibition can be used to regulate Ras membrane localization



Novel RCE1 inhibitors show specificity for RCE1 AAX cleavage




Hypothesis and specific aims

- ▶ I hypothesize that inhibition of RCE1 will be effective in treating pancreatic ductal adenocarcinoma with Ras mutations.

To test this hypothesis I propose the following specific aims:

- ▶ **Aim 1: Determine whether inhibition of RCE1 will decrease cell growth and viability of pancreatic ductal adenocarcinoma in vitro and in vivo.**
- ▶ **Aim 2: Determine whether RCE1 inhibition will have synergistic effects in combination with inhibitors of downstream effectors.**



Aim 1: Determine whether inhibition of RCE1 will decrease cell growth and viability of pancreatic ductal adenocarcinoma

8

I hypothesize the inhibition of RCE1 in pancreatic ductal adenocarcinoma will reduce Ras localization to the plasma membrane and Ras-dependent downstream phosphorylation, which will result in decreased pancreatic adenocarcinoma cell viability, migration and growth.

- ▶ Aim 1.1: Determine molecular effects of RCE1 inhibition on PDAC.
- ▶ Aim 1.2: Determine cellular effects of RCE1 inhibition on PDAC.

Aim 1.1: Determine molecular effects of RCE1 inhibition on PDAC.

- ▶ Ras localization to the membrane
 - ▶ Fluorescent confocal microscopy
 - ▶ Subcellular fractionation
- ▶ Ras - dependent downstream phosphorylation
 - ▶ ERK and Akt phosphorylation

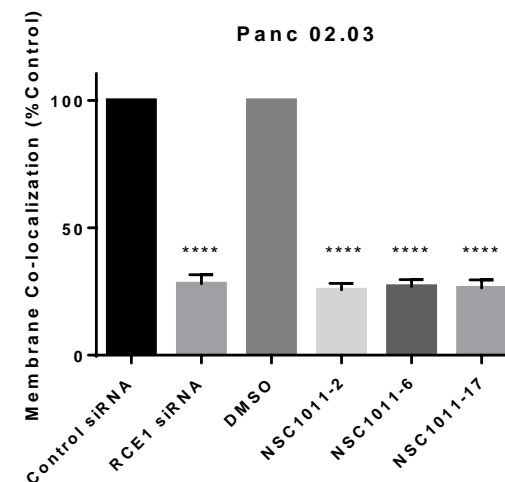
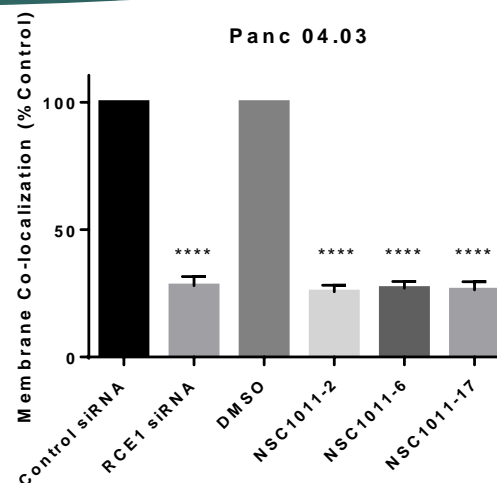
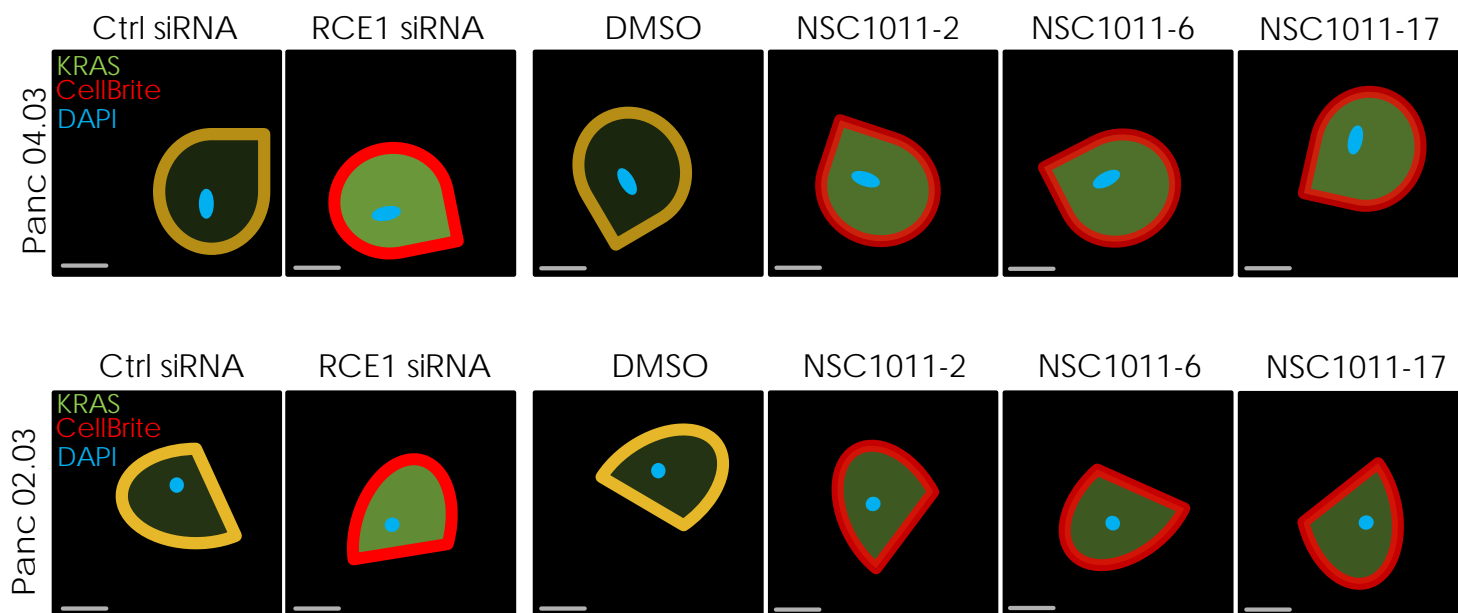


Cell lines

- ▶ Panc 02.03 and Panc 04.03
 - ▶ commercially available
 - ▶ possess K-Ras oncogenes
 - ▶ form tumors in nude or SCID mice
 - ▶ harvested in 1995 from the head-of-the-pancreas

Expected outcome: Ras localization to the membrane is impeded by RCE1 inhibition in PDAC cells

11

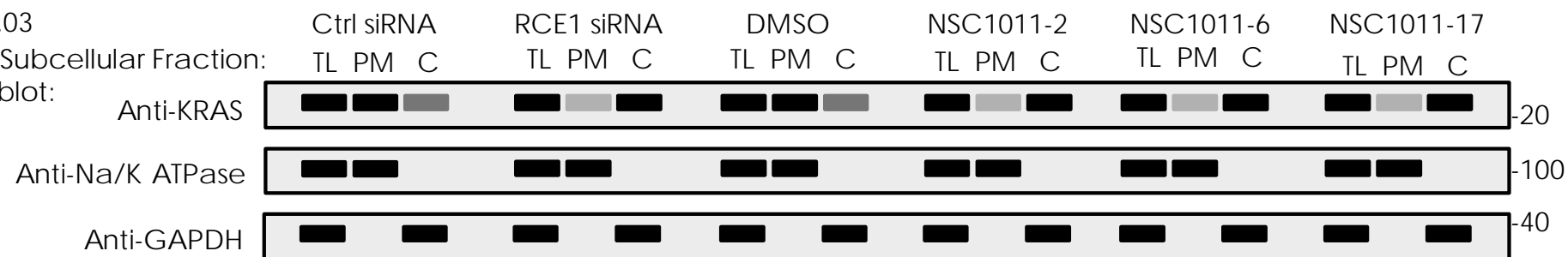


Expected outcome: Ras localization to the membrane is impeded by RCE1 inhibition in PDAC cells

12

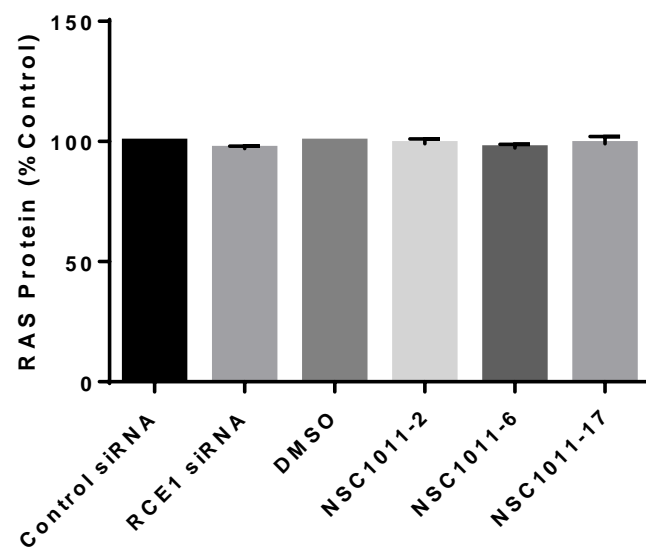
Panc 04.03/
Panc 02.03

Subcellular Fraction:
Immunoblot:

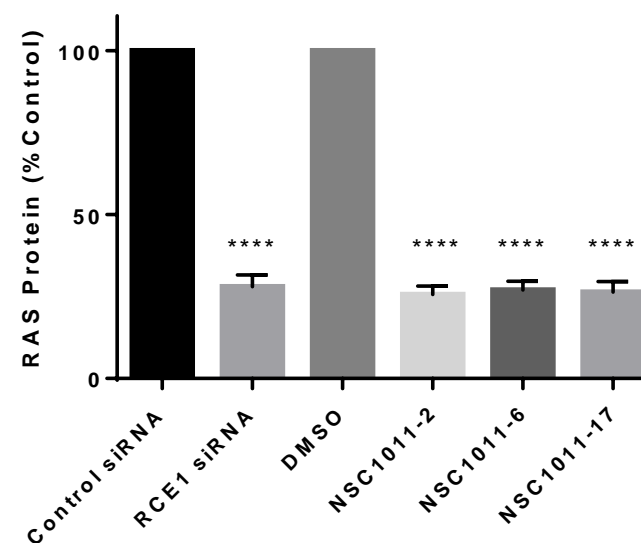


TL : Total Lysate
PM: Plasma Membrane
C: Cytosolic

Total Fraction



Membrane Fraction

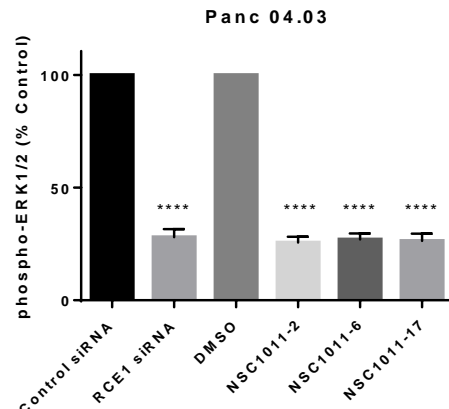
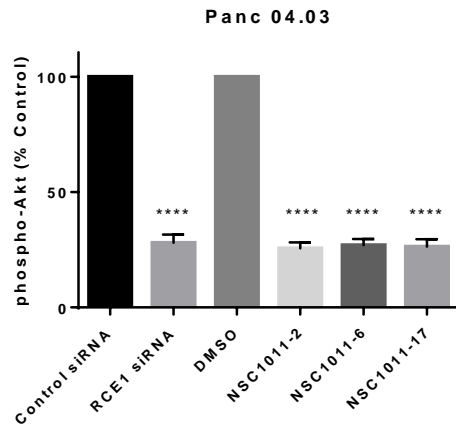
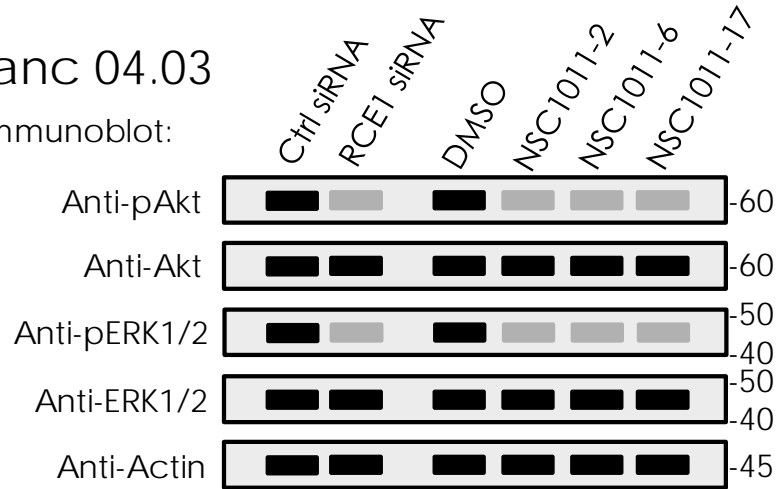


Expected outcome: Ras - dependent phosphorylation prevented by RCE1 inhibition in PDAC cells

13

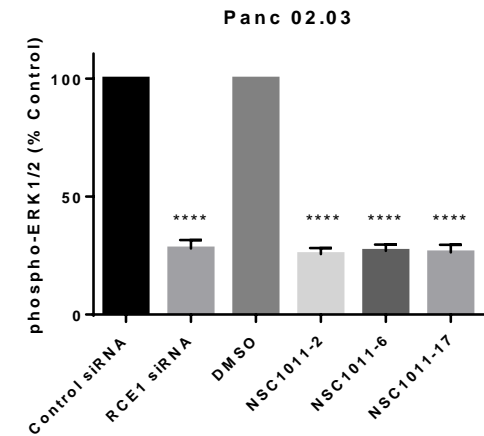
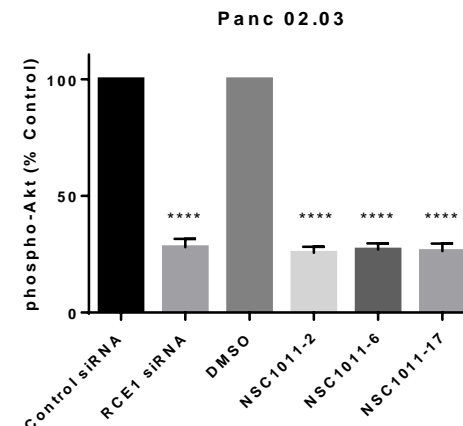
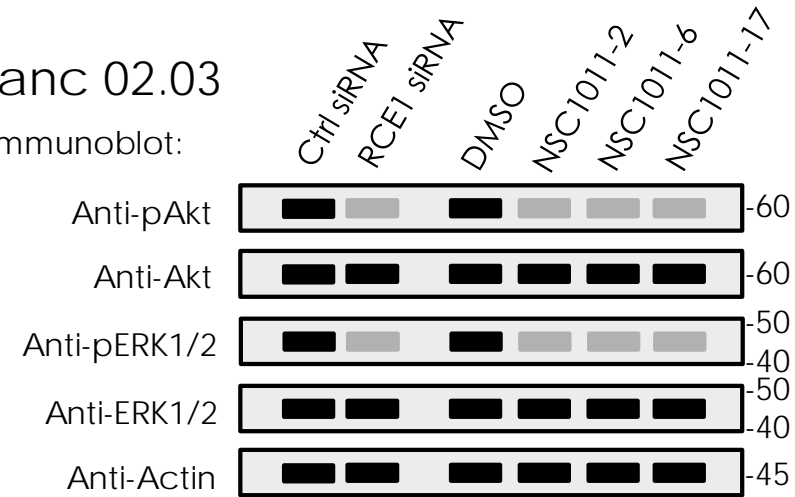
Panc 04.03

Immunoblot:



Panc 02.03

Immunoblot:





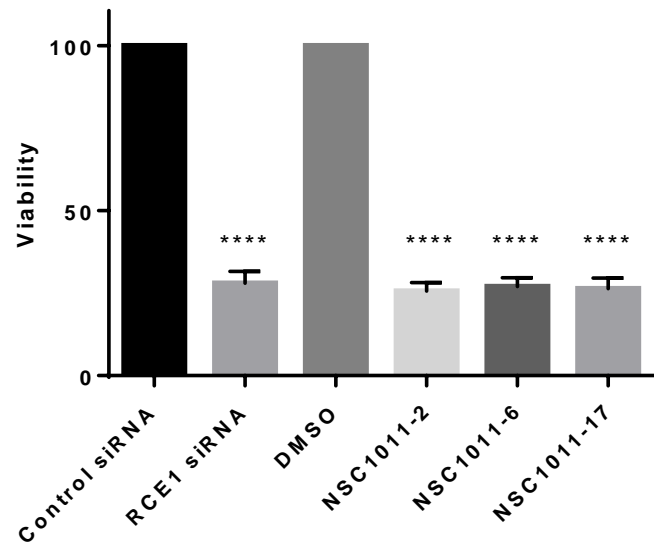
Aim 1.2: Determine cellular effects of RCE1 inhibition on PDAC.

- ▶ Viability and cell growth
 - ▶ Trypan blue assay
- ▶ Migration and Invasion
 - ▶ Scratch Test
 - ▶ Matrix Invasion Assay
- ▶ In vivo tumor burden, metastasis and survival

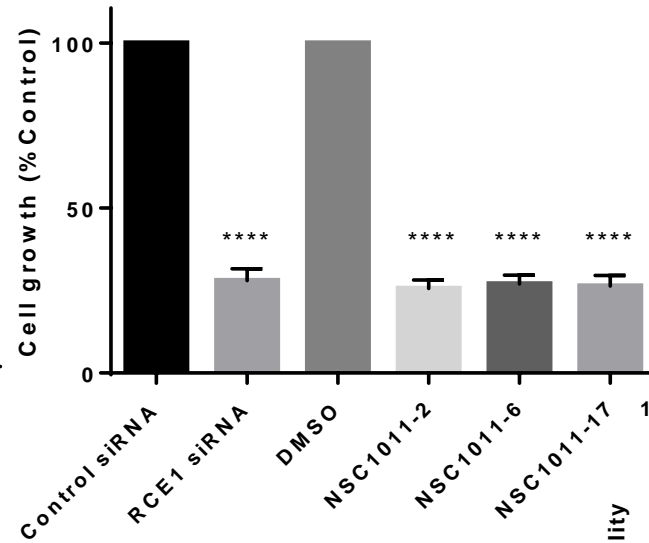
Expected outcome: RCE1 inhibition decreases viability and cell growth in PDAC cell

15

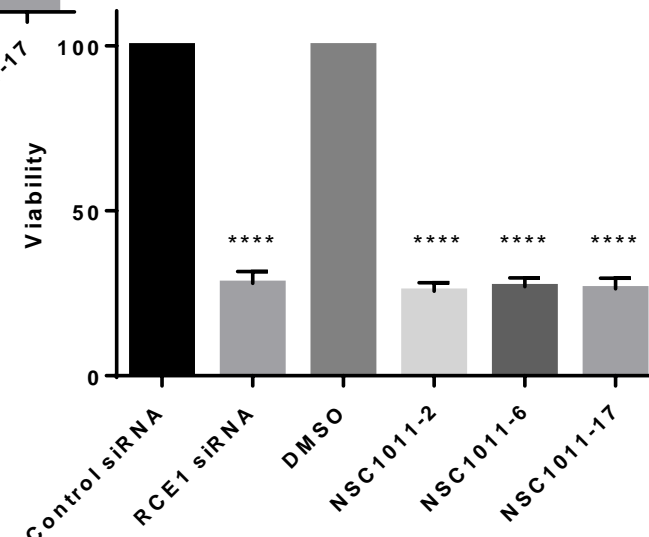
Panc 04.03



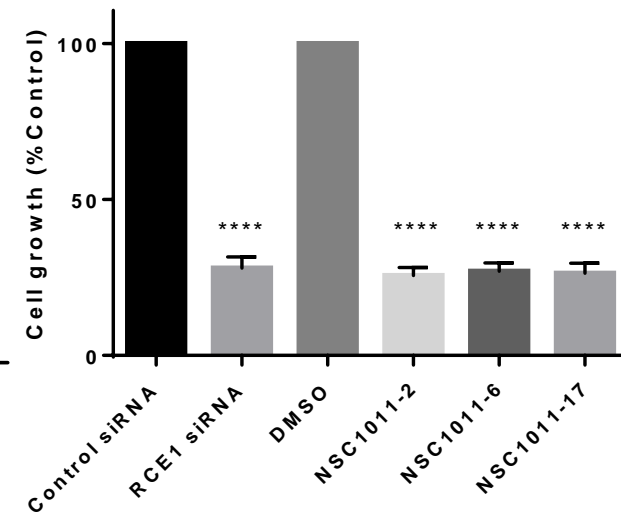
Panc 04.03



Panc 02.03

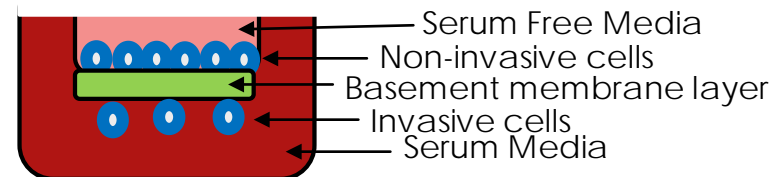
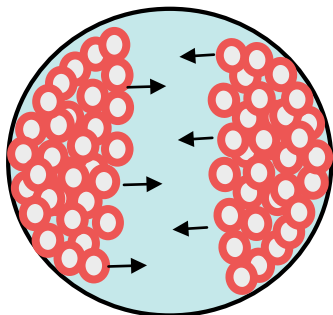


Panc 02.03

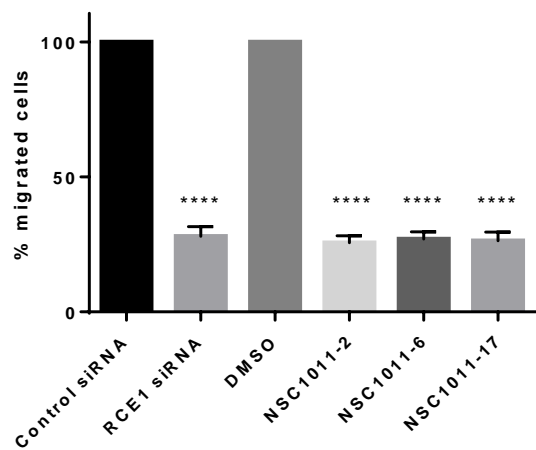


Expected Outcome: RCE1 inhibition decreases migration and invasiveness of PDAC cells

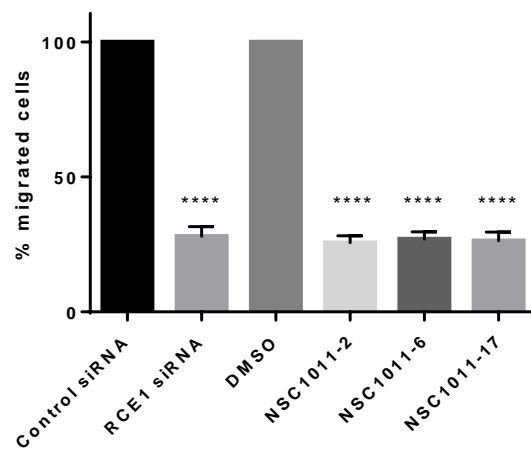
16



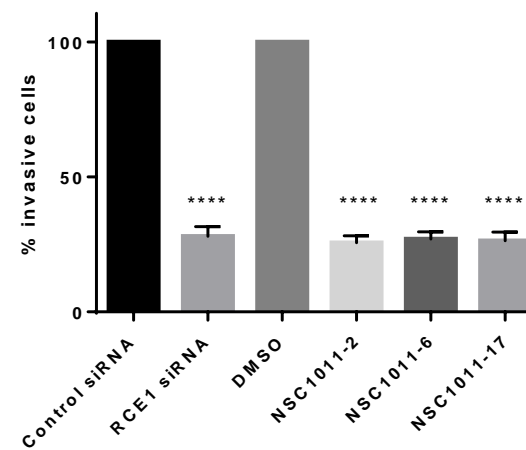
Panc 04.03



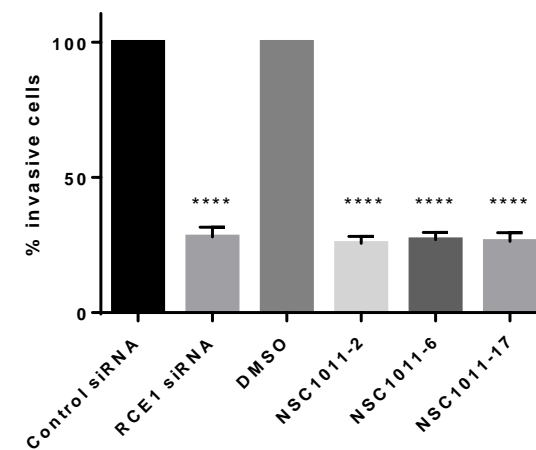
Panc 02.03



Panc 04.03



Panc 02.03



Expected outcome: Rce1 inhibition decreases tumor burden, metastasis and increases survival in PDAC mouse models

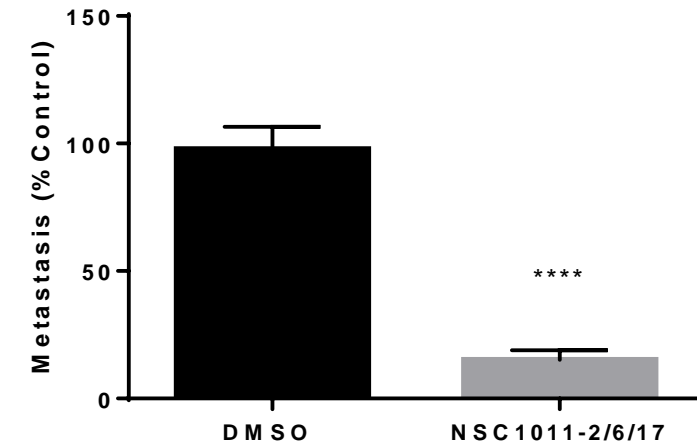
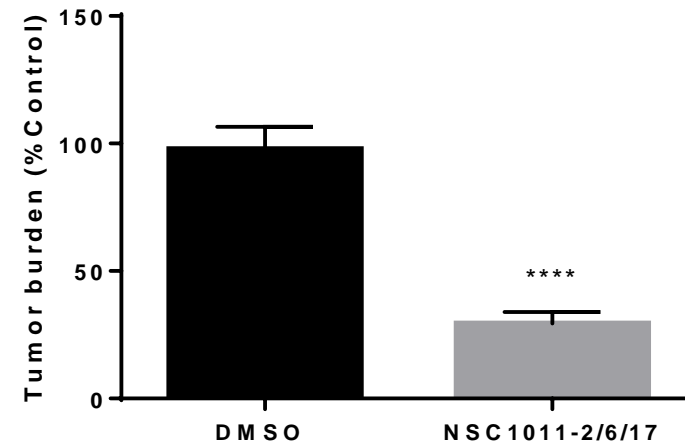
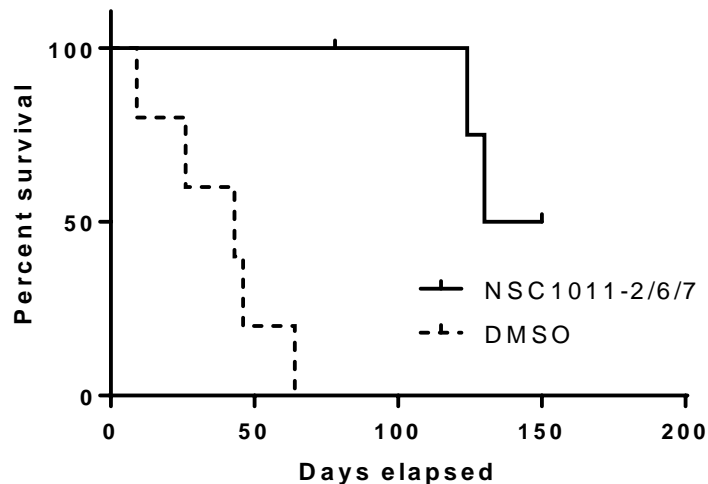
Luciferase Tagged
Panc04.03/
Panc02.03

Engraftment

DMSO

Visualization by luciferin
bioluminescence

NSC1011-2/6/17



Aim 1 - Pitfalls/Alternative approach:

- ▶ If this is not seen in vitro, alternate time points and concentrations will be determined by time curve and dose response, respectively.
- ▶ If this is not seen in vivo, I will alter the concentration and dosing schedule of the inhibitor injections.
- ▶ If this is still not seen, RNASeq and CyTOF will be performed to identify which genes or proteins that may be acting as compensatory mechanisms
- ▶ Tumor burden and metastasis can be determined postmortem.
- ▶ Cell line derived orthotopic xenograft “humanized” NOD/SCID mouse models or PDX-1-Cre, LSL-Kras^{G12D}, LSL-Trp53^{R172H/-} (KPC) genetically engineered mouse models (GEMMs) can be used
- ▶ Tumors can be harvested to determine the efficacy of RCE1 inhibition *in vivo*



Aim 2: Determine synergistic effects of targeting RCE1 in combination with inhibitors of downstream effectors in PDAC

I hypothesize that RCE1 inhibition will have synergistic effects in combination with downstream inhibitors, such as MEK, Akt or PI3K inhibitors, in pancreatic ductal adenocarcinoma.

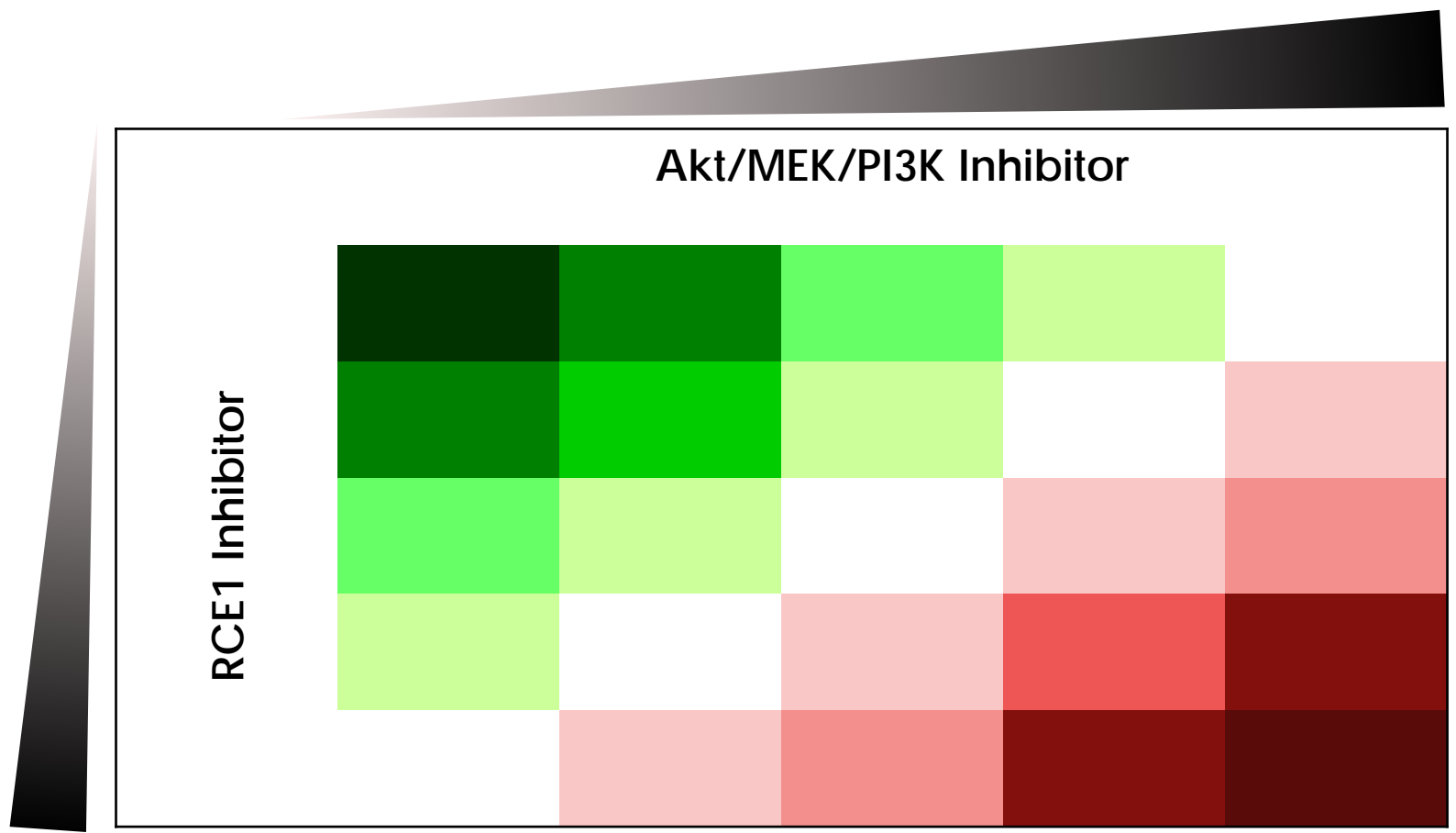
- ▶ Aim 2.1: Determine synergistic effects of RCE1 inhibitors in combination with MEK, Akt or PI3K inhibitors
- ▶ Aim 2.2: Evaluate effects of RCE1 inhibition in combination with MEK, Akt, PI3K inhibition on migration, invasion and in vivo tumor burden, metastasis and survival.

Aim 2.1: Determine synergistic effects of RCE1 inhibitors in combination with MEK, Akt or PI3K inhibitors

- ▶ Combination dose response assays for viability and cell growth in PDAC cells



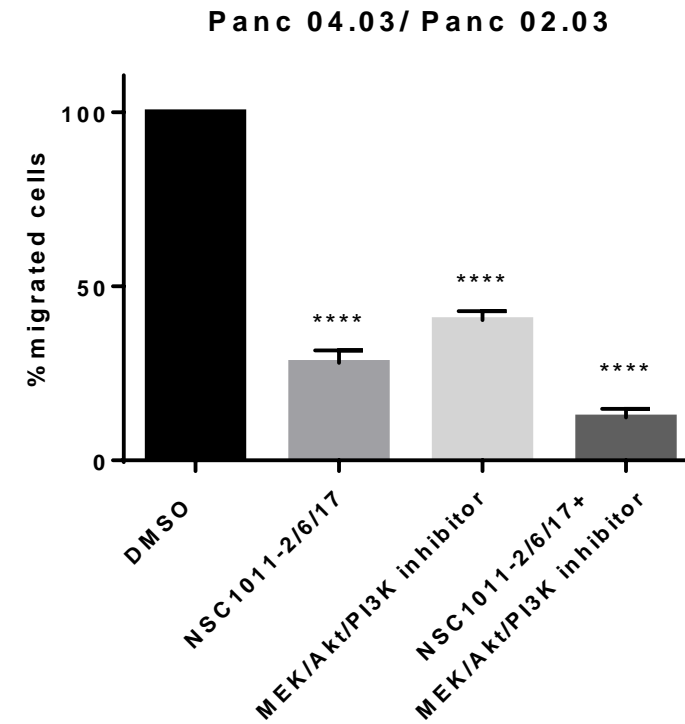
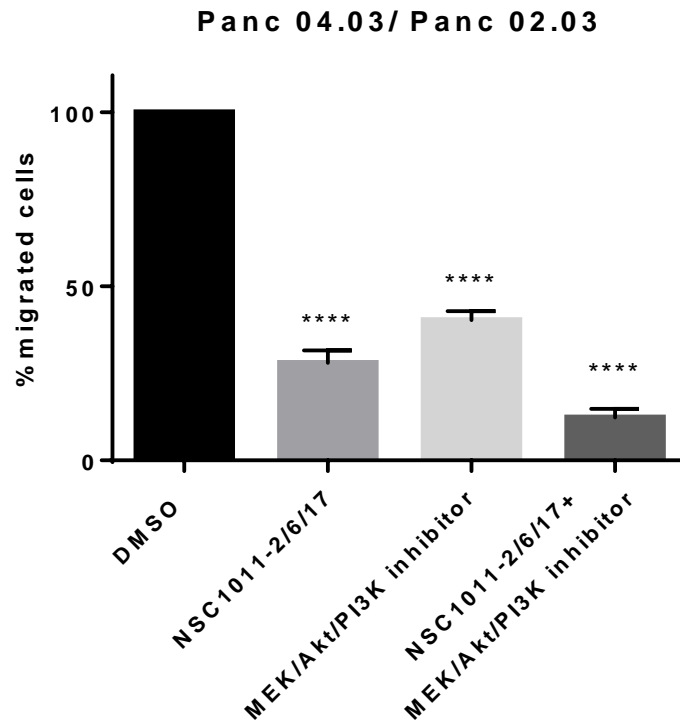
Expected outcome: Combined RCE1 and MEK, Akt or PI3K inhibition decreases viability and cell growth in PDAC cells



Aim 2.2: Evaluate effects of combined inhibition on migration, invasion and in vivo tumor burden, metastasis and survival.

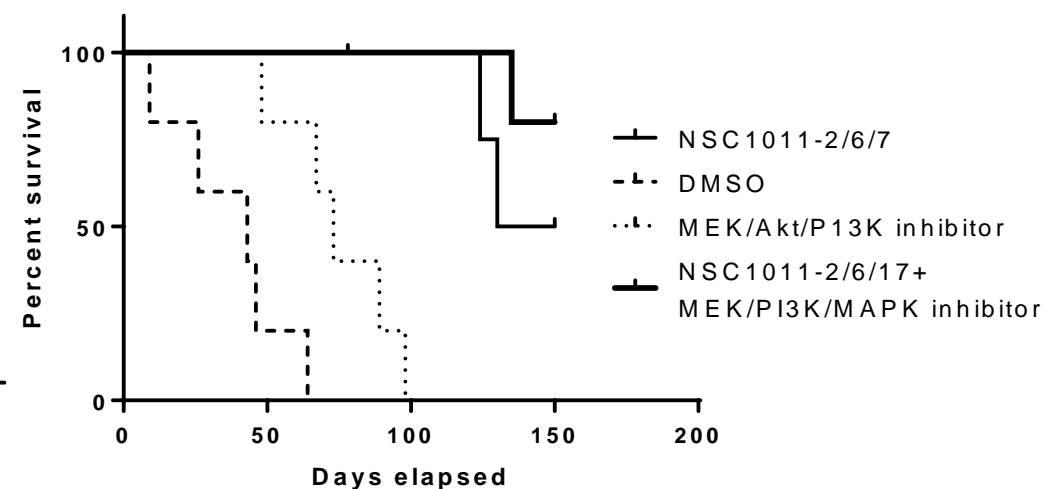
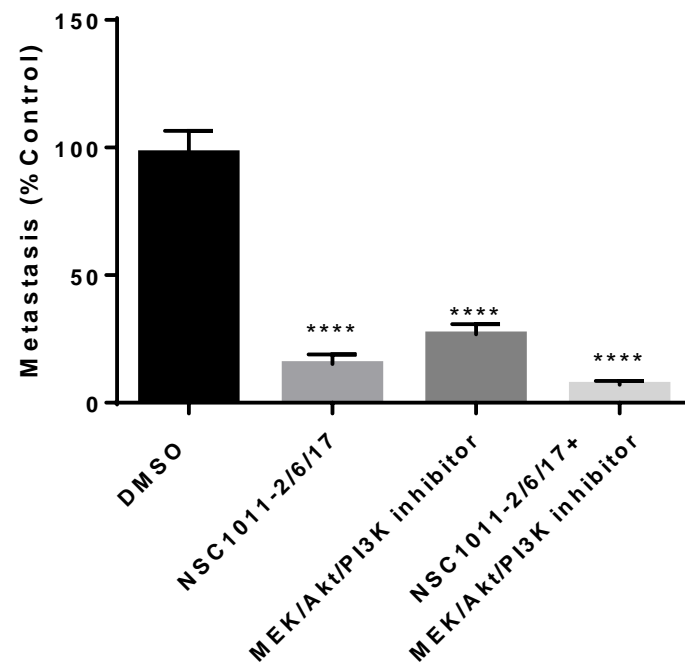
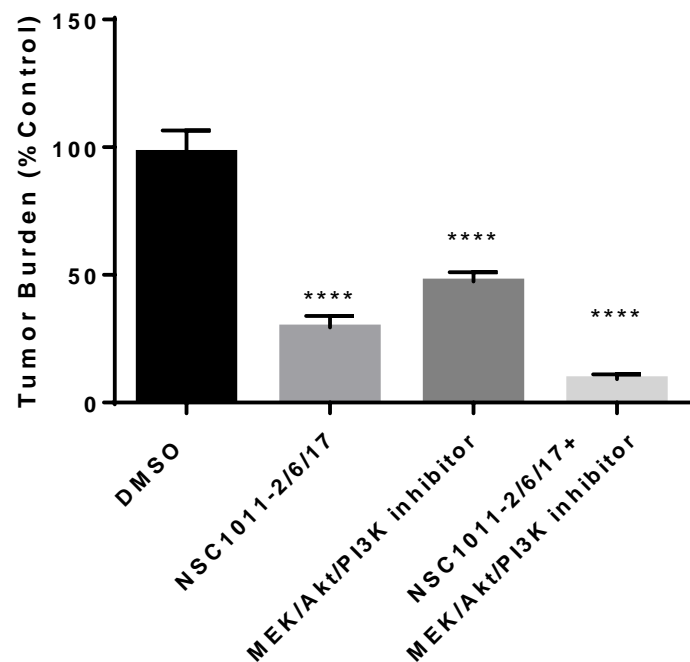
- ▶ Migration and Invasion
 - ▶ Scratch Test
 - ▶ Matrix Invasion Assay
- ▶ In vivo tumor burden, metastasis and survival

Expected Outcome: Combined RCE1 and MEK, Akt or PI3K inhibition decreases migration and invasiveness of PDAC cells



Expected outcome: Combined RCE1 and MEK, Akt or PI3K inhibition increases survival in mouse models

24



Aim 2 - Pitfalls/Alternative approach:

- ▶ If this is not seen in vitro, alternate time points and concentrations of both inhibitors will be determined by time curve and dose response, respectively.
- ▶ If this is not seen in vivo, I will alter the concentration and dosing schedule of injections of both inhibitors.
- ▶ If this is still not seen, RNASeq and CyTOF will be performed to identify which genes or proteins may be acting as compensatory mechanisms.
- ▶ Tumor burden and metastasis can be determined postmortem.
- ▶ Cell line derived orthotopic xenograft “humanized” NOD/SCID mouse models or PDX-1-Cre, LSL-Kras^{G12D}, LSL-Trp53^{R172H/-} (KPC) genetically engineered mouse models (GEMMs) can be
- ▶ Tumors can be harvested to determine the efficacy of RCE1 inhibition *in vivo*

Conclusion

- ▶ RCE1 inhibition potentially be used as a treatment in Ras mutated PDAC, as Ras localization to the membrane is essential for Ras activity and RCE1-mediated proteolytic cleavage of the terminal -AAX motif is an necessary step of Ras membrane localization
- ▶ Combination treatments of RCE1 inhibitor with MEK, Akt or PI3K inhibitors can decrease the concentration of both inhibitors required for effective treatment and there for decrease off target effects and toxicities.











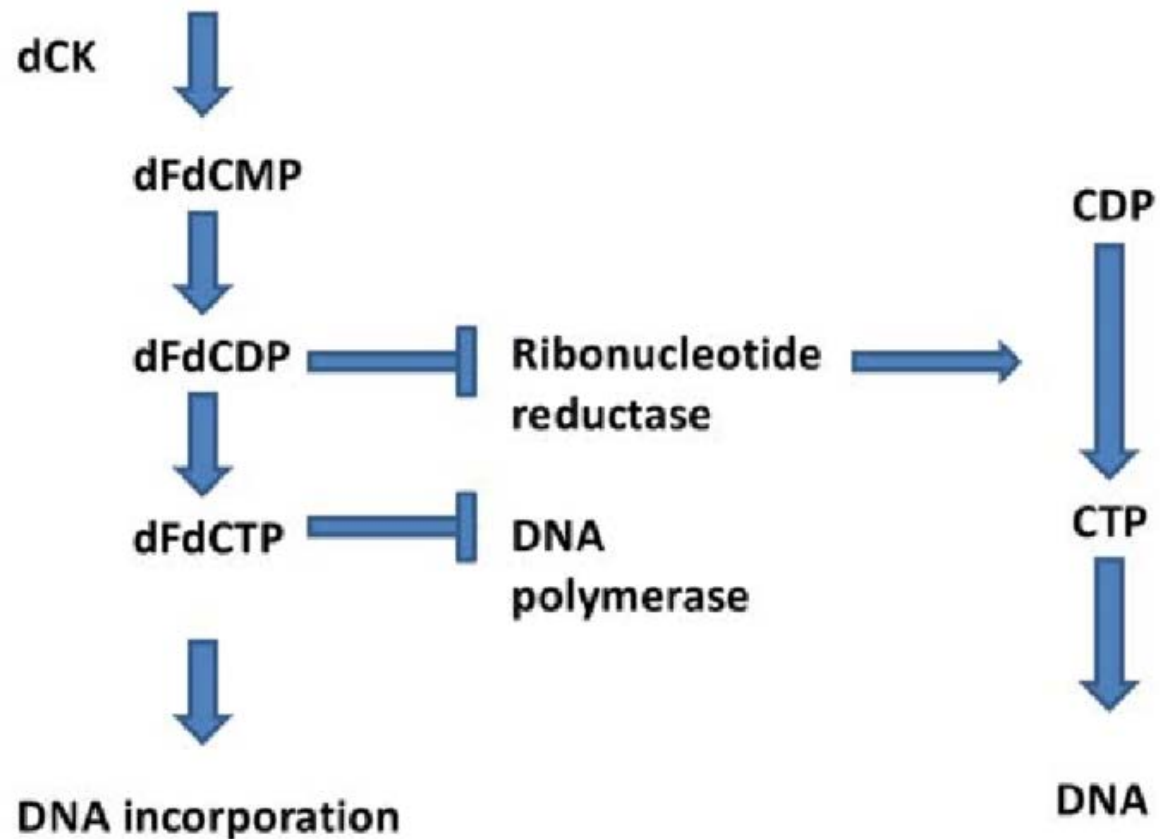






Gemcitabine

Gemcitabine (2', 2' difluorodeoxycytidine, dFdC)



Synthesis of Novel Rce1 Inhibitors

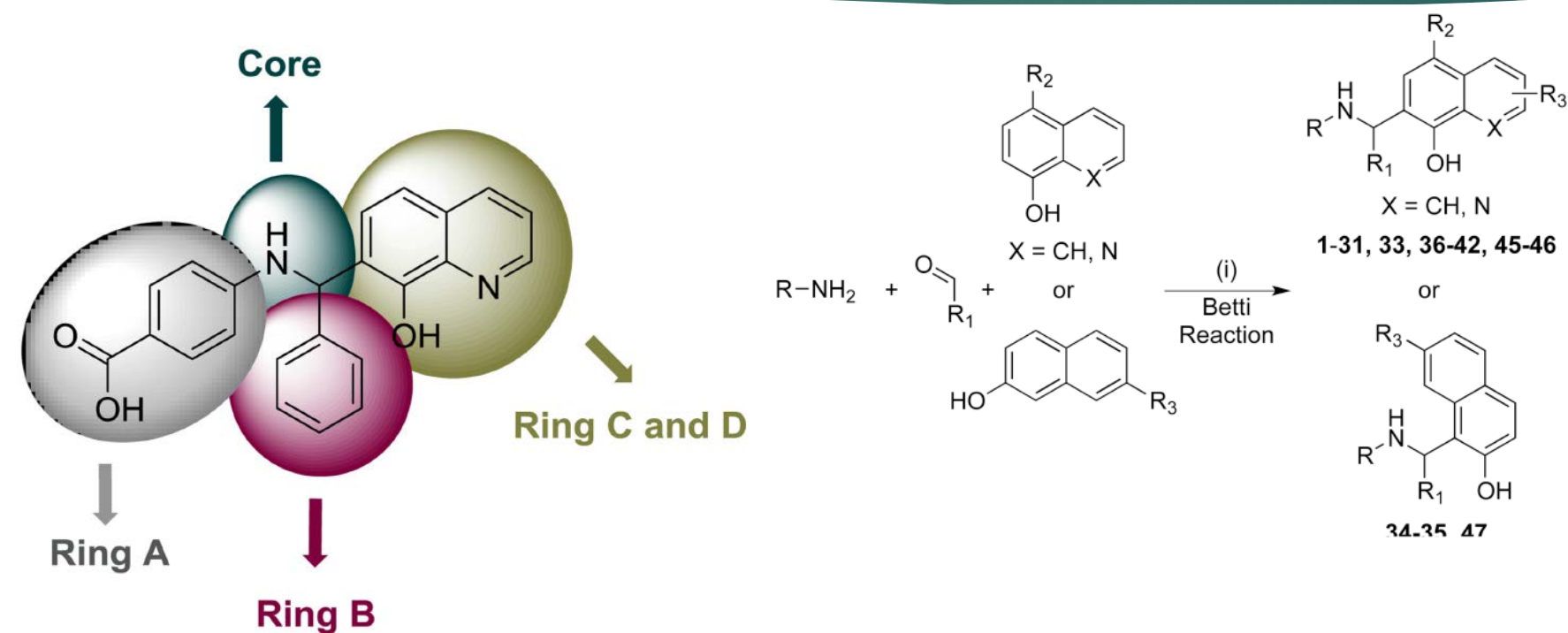
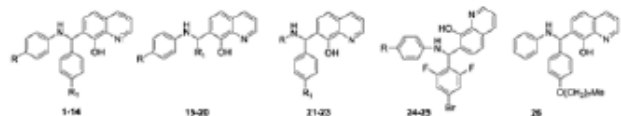
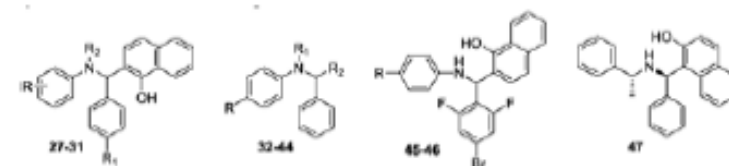


Figure 1. NSC1011 (1) and ring identification used to describe the SAR

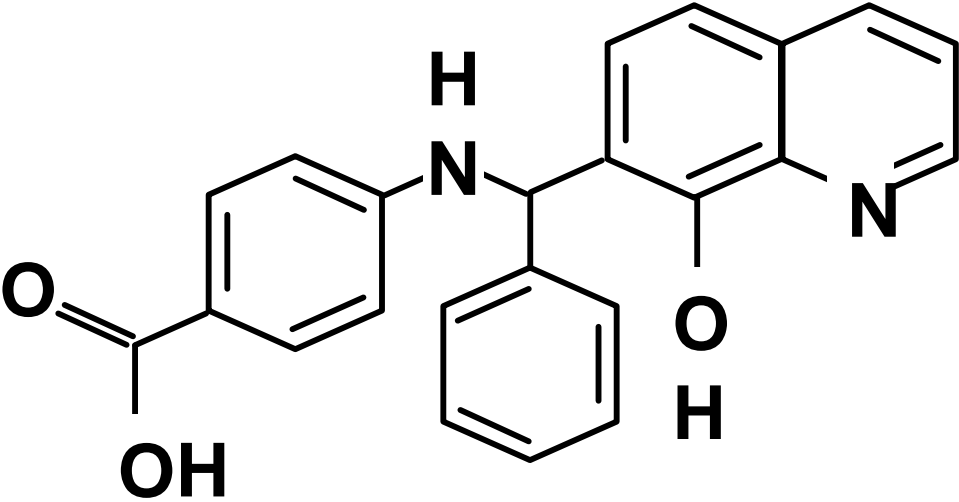


Enzymatic results for compounds 27-47

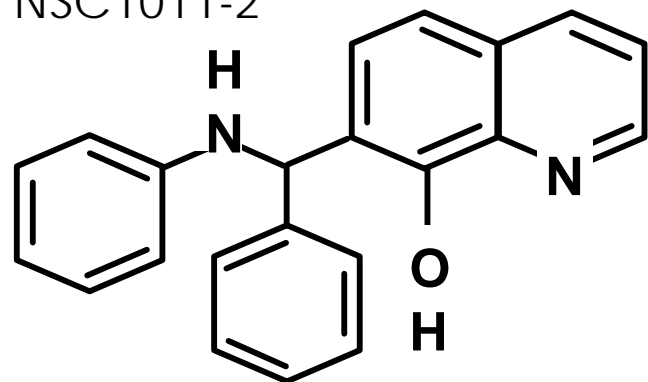


No.	R	R ₁	<i>HsRcel</i> Percent Activity Remaining ^a (10 μM)	<i>HsRcel</i> IC ₅₀ (μM) ^b
1 ^c	CO ₂ H	H	25 ± 0.05	6.9 ± 1.06
2	H	H	39 ± 1.3	8.9 ± 1.08
3	CO ₂ H	CN	76 ± 3.1	16 ± 1.1
4	CO ₂ H	Me	32 ± 3.6	7.1 ± 1.0
5	CO ₂ H	Br	23 ± 1.3	6.7 ± 1.1
6	CO ₂ H	F	32 ± 0.01	8.2 ± 1.1
7 ^d	H	NO ₂	85 ± 5.0	11 ± 1.2
8 ^d	Me	H	54 ± 5.2	8.8 ± 1.1
9	CN	H	56 ± 0.2	nd
10	NO ₂	Br	64 ± 1.2	nd
11	CO ₂ Et	H	52 ± 3.2	nd
12	CO ₂ Et	CF ₃	89 ± 3.8	nd
13	CO ₂ Et	F	63 ± 0.4	nd
14	CO ₂ Et	Cl	71 ± 0.6	nd
15	CO ₂ H	2-pyridine	90 ± 5.2	38 ± 1.1
16	CO ₂ H	3-pyridine	70 ± 1.8	14 ± 1.0
17	CO ₂ H	cyclohexyl	34 ± 4.8	9.8 ± 1.1
18	H	cyclohexyl	59 ± 0.03	nd
19	CO ₂ Et	cyclopentyl	82 ± 1.2	nd
20	H	H	82 ± 0.49	nd
21		H	83 ± 5.7	nd
22	Ph(CO)	H	81 ± 1.1	nd
23	PhCH	H	73 ± 2.1	nd
24	H	-	76 ± 1.7	nd
25	CO ₂ H	-	64 ± 3.7	nd
26	-	-	89 ± 3.5	nd

No.	R	R ₁	R ₂	<i>HsRcel</i> Percent Activity Remaining (10 μM) ^a	<i>HsRcel</i> IC ₅₀ (μM) ^b
27	H	H	H	17 ± 0.4	4.9 ± 1.1
28	<i>g-t</i> -butyl	H	H	38 ± 0.47	5.0 ± 1.1
29	<i>p</i> -CO ₂ H	H	H	19 ± 2.4	4.2 ± 1.1
30	<i>m</i> -CO ₂ H	H	H	23 ± 5.5	5.3 ± 1.1
31	<i>p</i> -CO ₂ H	Br	H	23 ± 1.1	3.9 ± 1.0
32	CO ₂ Me	H		65 ± 2.1	nd
33	H	H		27 ± 3.2	5.4 ± 1.1
34	H	H		59 ± 0.65	nd

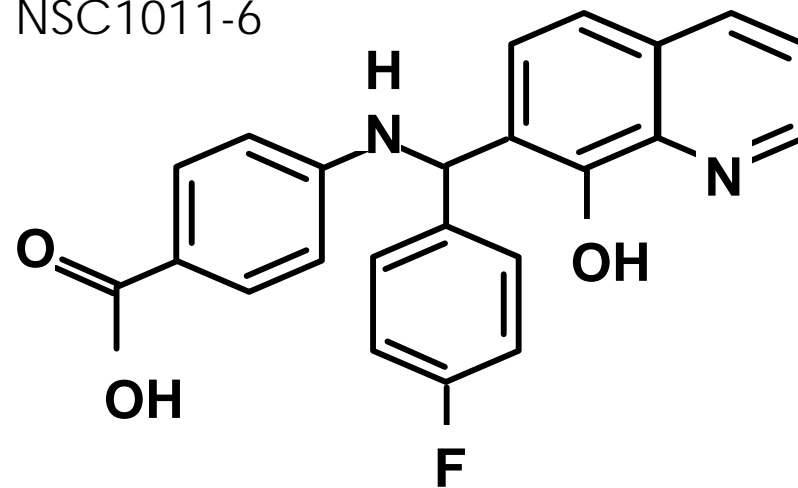


NSC1011-2



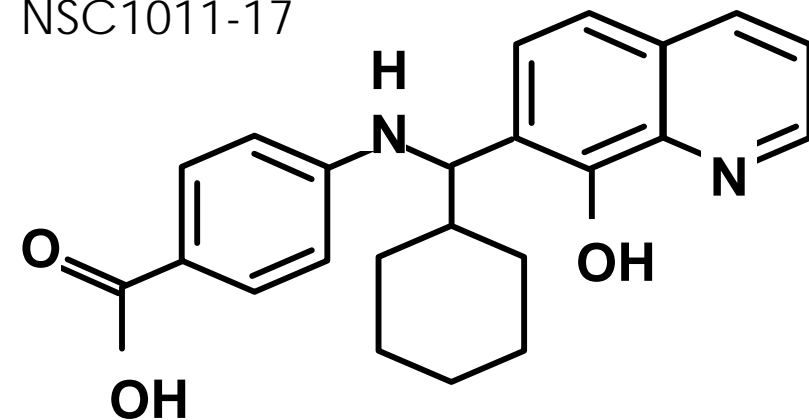
RCE1 IC₅₀: 8.9 ± 1.08 μM

NSC1011-6



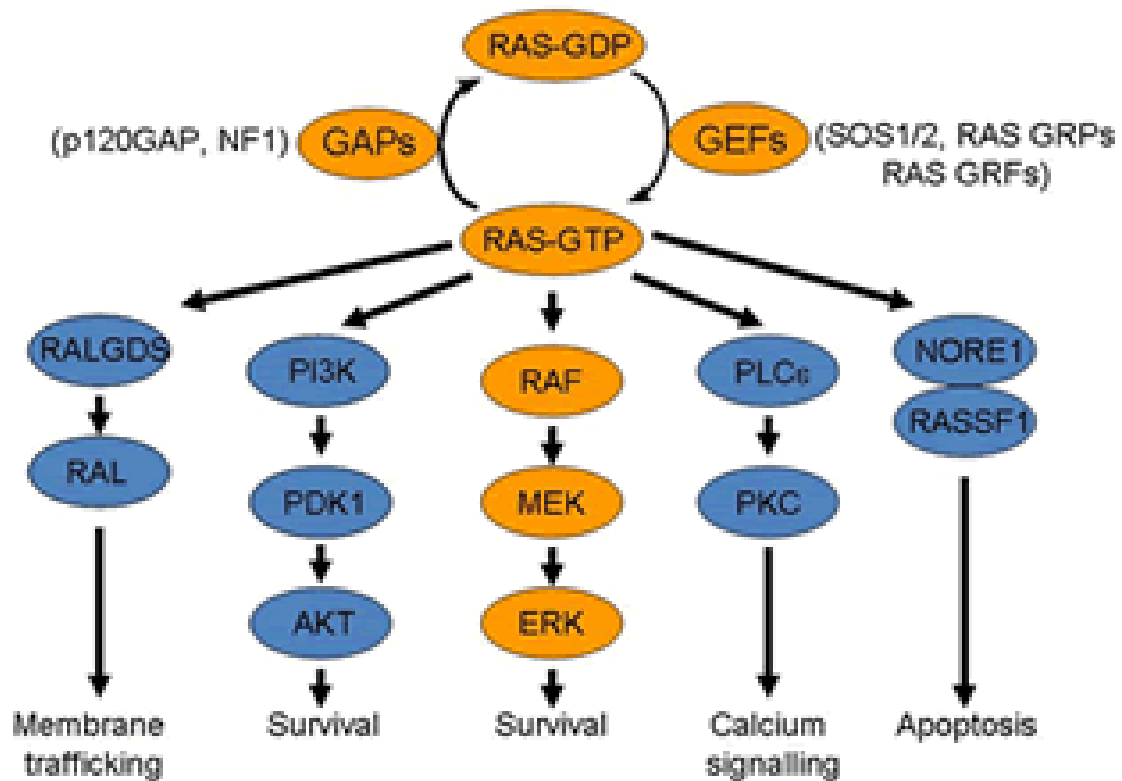
8.2 ± 1.1 μM

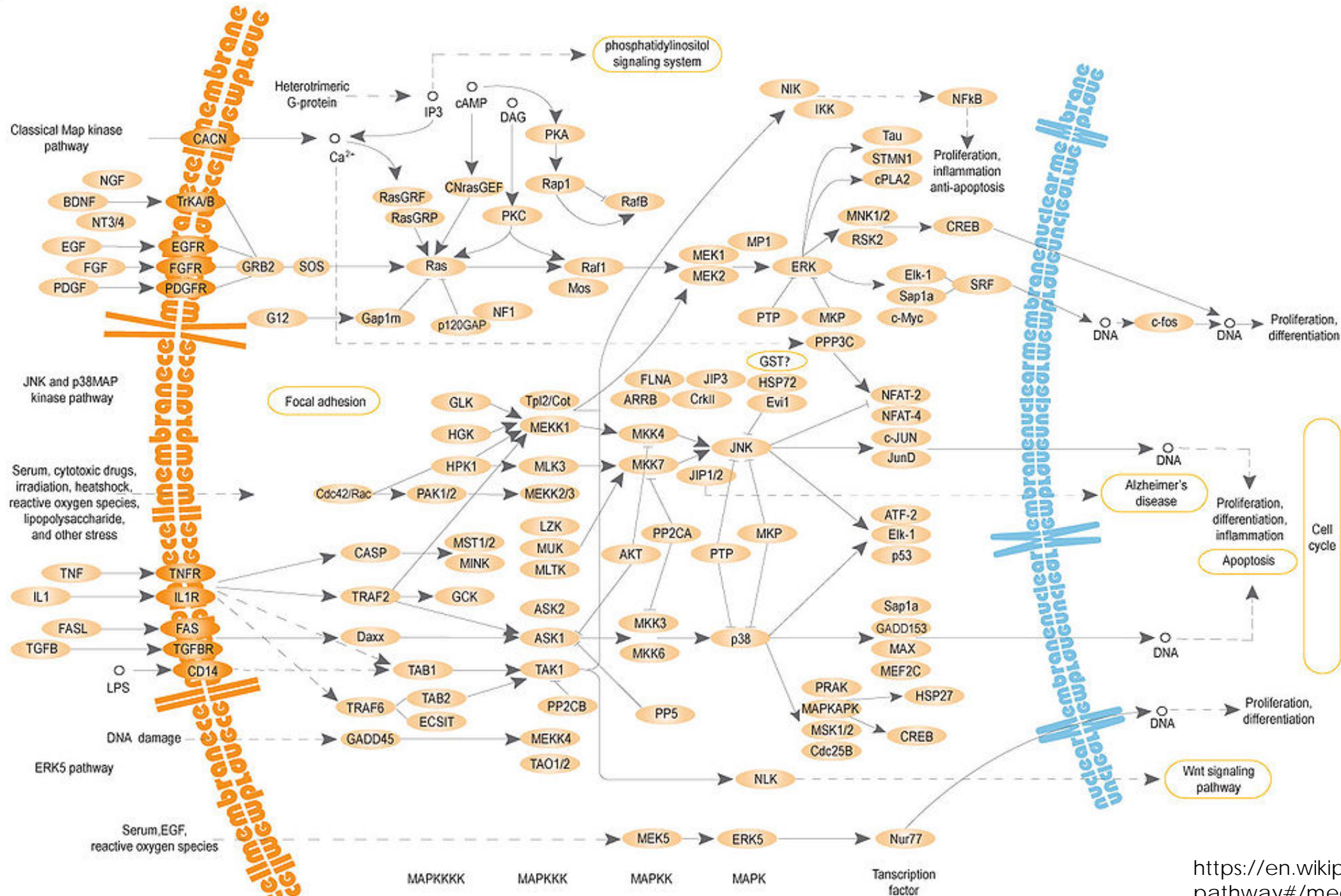
NSC1011-17

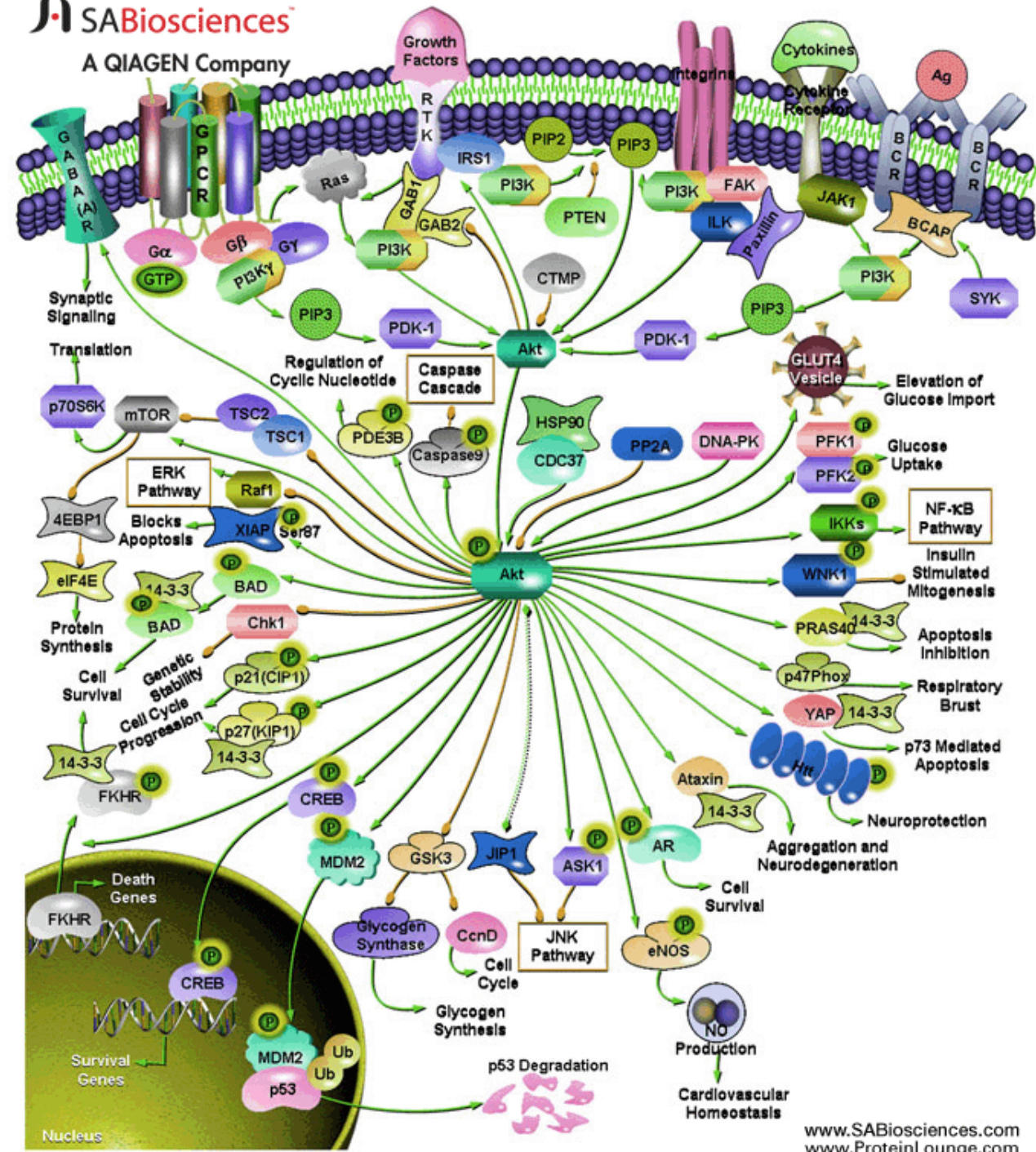
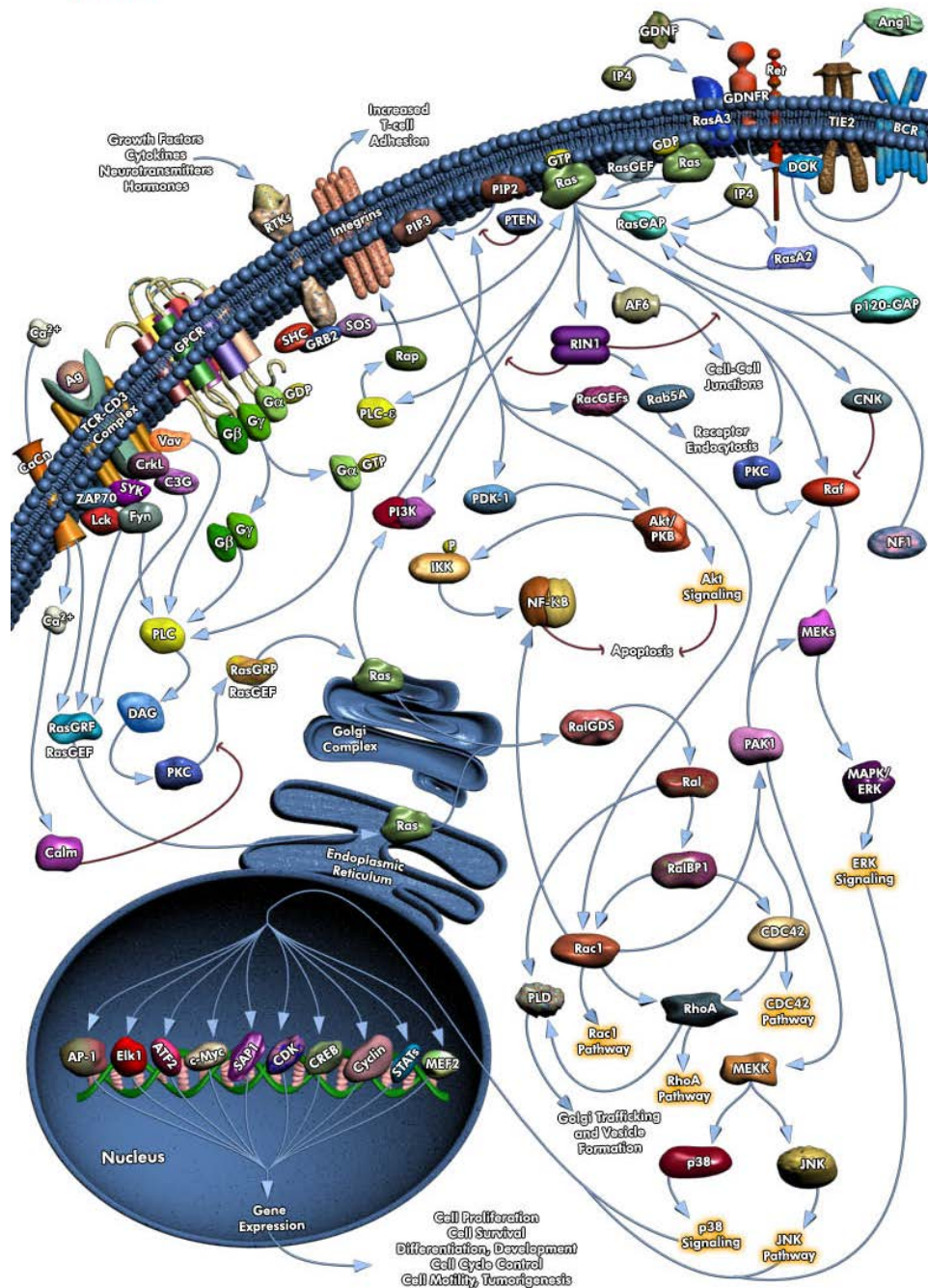


9.8 ± 1.1 μM

RAS pathway



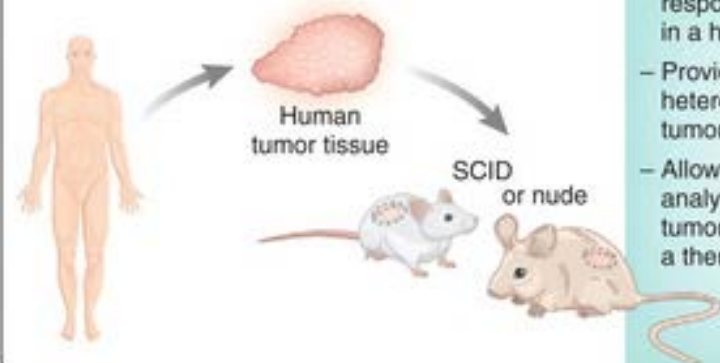




ADVANTAGES

DISADVANTAGES

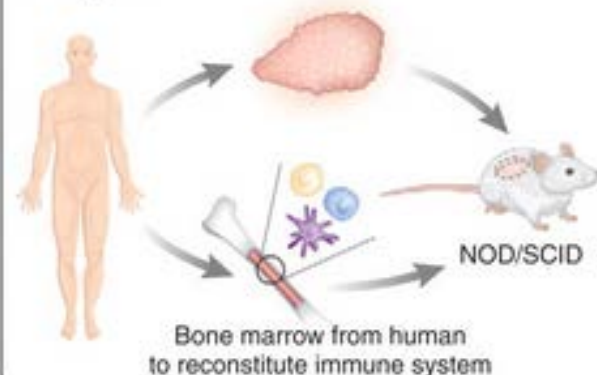
Orthotopic xenograft of human tumors



- Can predict the drug response of a tumor in a human patient
- Provides realistic heterogeneity of tumor cells
- Allows for rapid analysis of human tumor response to a therapeutic regime

- Mice are immunocompromised, providing a less realistic tumor microenvironment

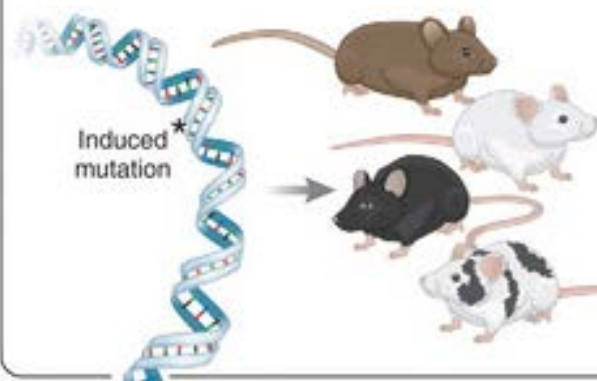
Xenograft of human tumor in humanized mice



- Appropriately mimics human tumor microenvironment
- Can predict the drug response of a tumor in a human patient
- Provides realistic heterogeneity of tumor cells

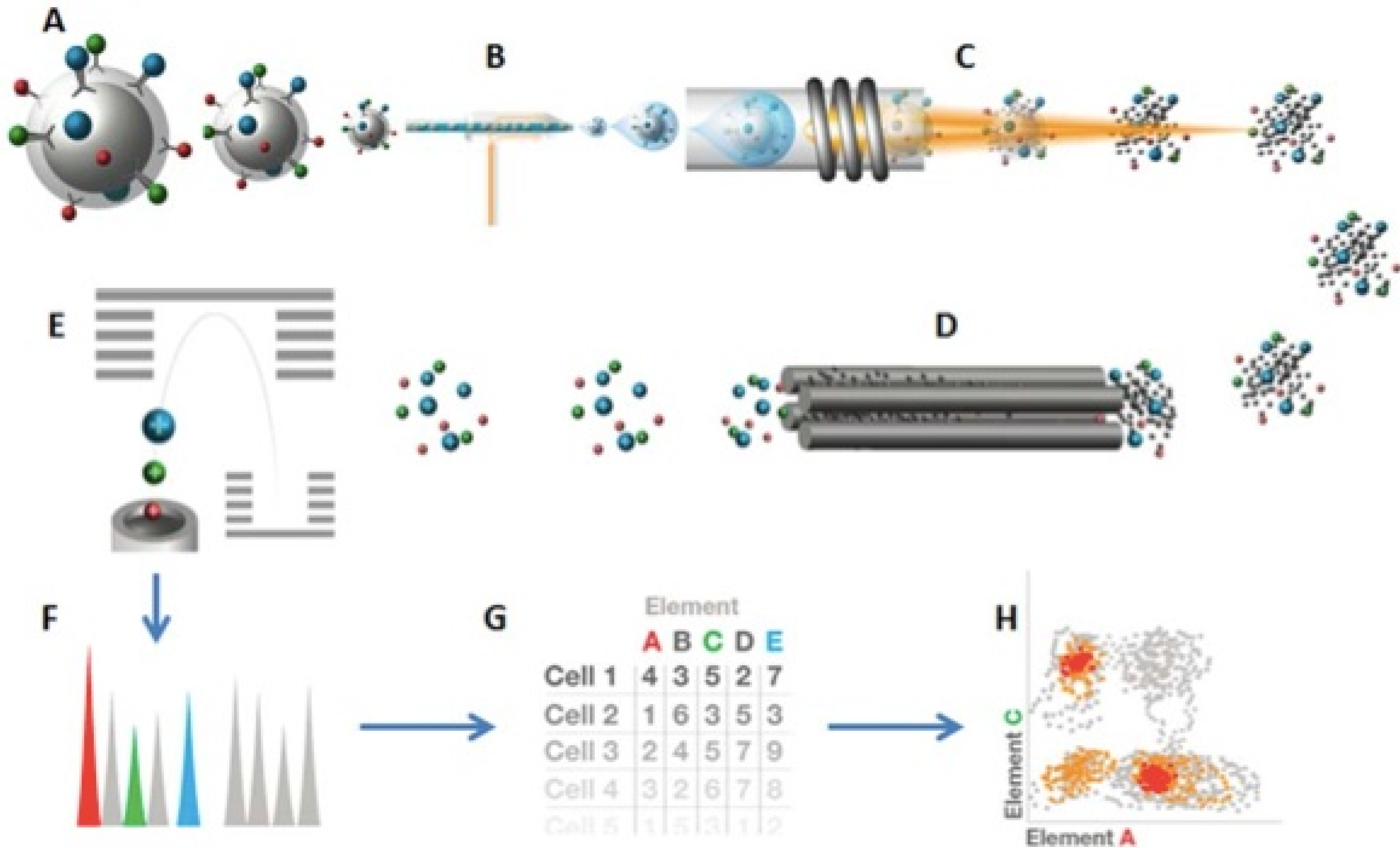
- Expensive
- Technically complicated

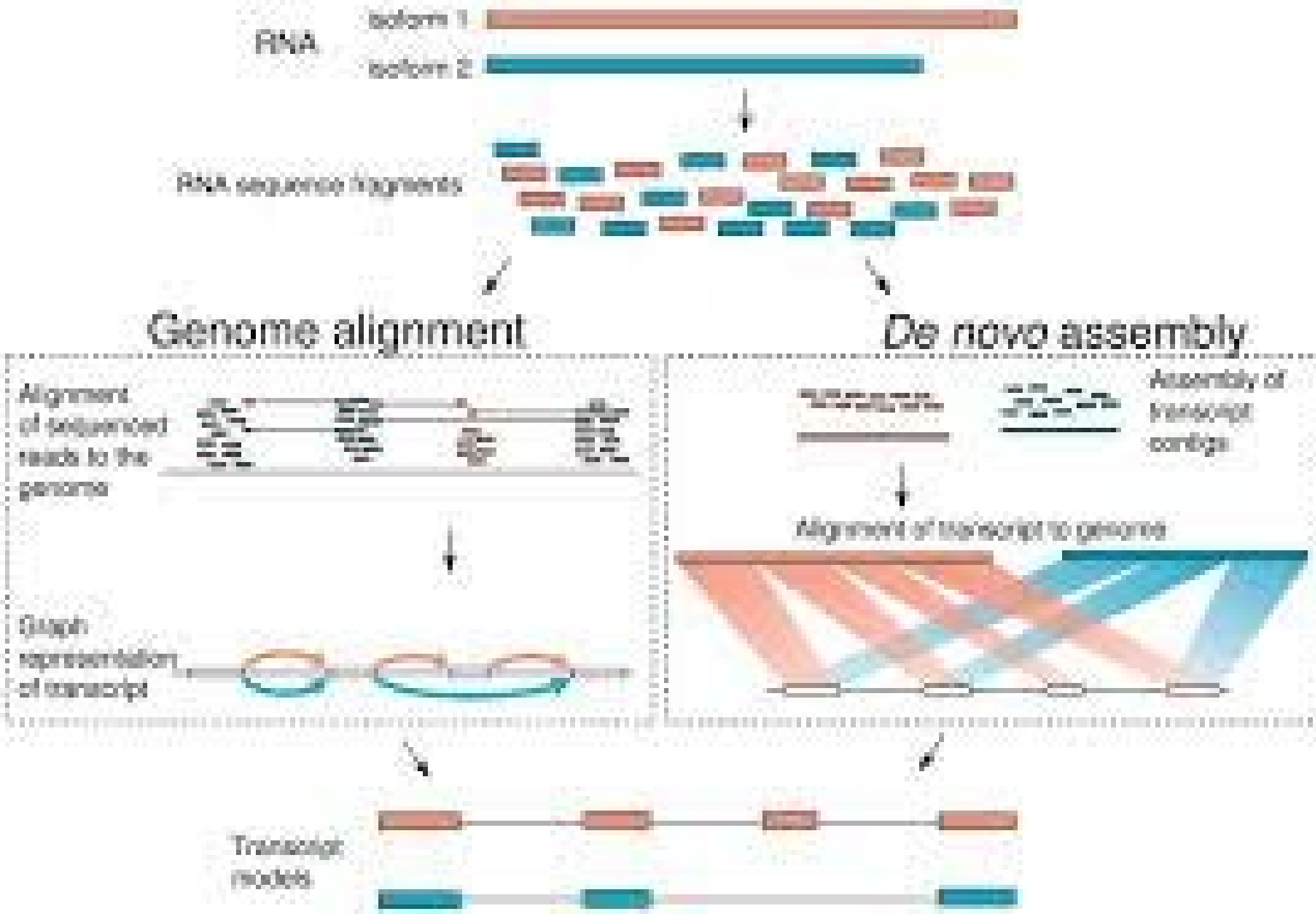
Genetically engineered mice (GEM)



- Potential analysis of many genetic backgrounds by using a variety of mouse strains
- Tumor exists in the presence of competent immune system (realistic microenvironment)
- Defined mutations can mimic those identified in human tumors
- Can follow tumor development from early time points

- Targets a limited number of genes which is usually not reflective of the complex heterogeneity of human tumor cells
- Development is costly and time consuming, often requiring years of work before validation
- Tumor development in animals is slow and variable





Alleles	Phenotype	Metastasis	Survival	Comments
⁷⁸ Elastase-CreERT;K-ras ^{G12D}	mPanIN	No	<18 months	Acinar derived mPanINs
⁷⁹ Elastase-Tgfa	Acinar to ductal metaplasia, mPanINs, fibrosis	No	<12 months	
⁸⁰ Elastase-tTA TRE-Cre;K-ras ^{G12V}	mPanINs, PDAC	No	18 months	PDAC development in adult mice through pancreatitis, inducible
⁸¹ Pdx1-Cre;Lkb1 ^{lox/lox}	Mucinous cystadenoma	No	2.5 months	
¹⁸ Pdx1-Cre;K-ras ^{G12D}	mPanIN to PDAC progression	Yes	>12 months	Slow progression
⁸¹ Pdx1-Cre;K-ras ^{G12D} ;Lkb1 ^{lox/lox}	mPanIN, PDAC	N/A	4.5 months	PDAC, accelerated
⁸² Pdx1-Cre;K-ras ^{G12D} ;Brca2 ^{Tr/D11}	mPanIN to PDAC progression	N/A	N/A,	PDAC, accelerated, some sarcomatoid cancers
⁸³ Pdx1-Cre;K-ras ^{G12D} ;Ink4a/Arf ^{-/-}	mPanIN to PDAC progression	Yes	5 months	PDAC, short latency
⁸³ Pdx1-Cre;K-ras ^{G12D} ;Ink4a/Arf ^{-/-} ;p53 ^{lox/lox}	Rapid mPanIN to PDAC progression	Yes	2 months	PDAC, short latency
⁸³ Pdx1-Cre;K-ras ^{G12D} ;Ink4a/Arf ^{+/-}	mPanIN to PDAC progression	Yes	10 months	Slow progression, macrometastatic
¹⁶ Pdx1-Cre;K-ras ^{G12D} ;Ink4a/Arf ^{lox/lox}	Rapid mPanIN to PDAC progression	(Yes)	2 months	Micrometastases, partly undifferentiated tumors
⁸¹ Pdx1-Cre;K-ras ^{G12D} ;p21 ^{+/-}	mPanIN to PDAC progression	N/A	2.5 months	PDAC, accelerated
⁸¹ Pdx1-Cre;K-ras ^{G12D} ;p53 ^{R270H/+} ;Rac1 ^{lox/lox}	Reduced mPanINs	No	N/A	Extended survival, delayed mPanIN development
⁸³ Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox}	mPanINs, PDAC, rapid progression	No	3 months	PDAC & cystic tumors, short latency
⁸⁴ Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox} ;Brca1 ^{lox/FH-126A}	mPanIN to PDAC progression	N/A	N/A	No acceleration of Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox} phenotype
⁸⁴ Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox} ;Brca1 ^{lox/S1598S}	mPanIN to PDAC progression	N/A	45 days	PDAC, accelerated, cystic tumors
⁸² Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox} ;Brca2 ^{D11/D11}	mPanIN to PDAC progression	N/A	300 days	
⁸⁴ Pdx1-Cre;K-ras ^{G12D} ;p53 ^{lox/lox} ;Brca1 ^{lox/lox}	mPanIN to PDAC progression	N/A	40 days	PDAC, accelerated, cystic tumors
⁸⁵ Pdx1-Cre;p53 ^{lox/lox} ;Brca2 ^{lox/lox}	Various histologies including PDAC	N/A	300 days	Ductal and acinar carcinomas
²² Pdx1-Cre;K-ras ^{G12D} ;p53 ^{R172H/+}	mPanIN to PDAC progression	Yes	5 months	Well differentiated PDAC, some sarcomatoid tumors
⁸² Pdx1-Cre;K-ras ^{G12D} ;p53 ^{R270H/+} ;Brca2 ^{Tr/+}	mPanIN to PDAC progression	N/A	<5 months	PDAC, accelerated, some sarcomatoid tumors
⁸² Pdx1-Cre;K-ras ^{G12D} ;p53 ^{R270H/+} ;Brca2 ^{Tr/D11}	mPanINs, PDAC & acinar cell carcinoma	Yes	2.5 months	Model of familial PDAC, short latency

82	Pdx1-Cre;K-ras ^{G12D} ;p53 ^{R270H/+} ;Brca2 ^{Tr/D11}	mPanINs, PDAC & acinar cell carcinoma	Yes	2.5 months	Model of familial PDAC, short latency
86	Pdx1-Cre;K-ras ^{G12D} ;Rb ^{lox/lox}	mPanIN to PDAC progression	No	3 months	PDAC, accelerated, trend towards cystic neoplasms
23 , 36	Pdx1-Cre;K-ras ^{G12D} ;SMAD4 ^{lox/lox}	IPMN to PDAC progression	Yes	9 months	Model of IPMN-derived PDAC
87	Pdx1-Cre;Lkb1 ^{lox/lox}	Acinar to ductal metaplasia, serous cystadenomas	No		Model of Peutz-Jeghers-Syndrome
78	Pdx1-CreERT;K-ras ^{G12D} ;R26NIC	Accelerated mPanIN development	N/A	N/A	PDAC with long latency
39	Pdx1-Cre;K-ras ^{G12D} ;Usp9x ^{lox/+}	Accelerated mPanIN to PDAC progression	N/A	N/A	Accelerated phenotype compared to Pdx1-Cre;K-ras ^{G12D}
88	Ptf1a-Cre;K-ras ^{G12D} ;β-catenin	Ductal and cribriform tumors	N/A	N/A	
18	Ptf1a-Cre;K-ras ^{G12D}	mPanIN to PDAC progression	Yes	>12 months	Slow progression
89	Ptf1a-Cre;K-ras ^{G12D} ;Ikk ^{lox/lox}	Reduced mPanINs	Yes	N/A	Extended survival, delayed mPanIN development
26	Ptf1a-Cre;K-ras ^{G12D} ;TGFβIR ^{lox/lox}	mPanIN to PDAC progression	Yes	2 months	Aggressive PDAC
90	Ptf1a-Cre;K-ras ^{G12D} ;Elastase-Tgfa	mPanINs& IPMNs progression to PDAC	Yes	7 months	IPMNs of pancreatobiliary subtype
91	Ptf1a-Cre;K-ras ^{G12D} ;MUC1.Tg	mPanIN to PDAC progression	Yes	N/A	PDAC, accelerated, metastatic
92	Ptf1a-Cre;K-ras ^{G12D} ;Notch1 ^{lox/lox}	mPanIN to PDAC progression	Yes	12 months	Slightly accelerated through loss of Notch 1
92	Ptf1a-Cre;K-ras ^{G12D} ;Notch2 ^{lox/lox}	MCNs, mPanIN1, progression to PDAC	Yes	>15 months	Sarcomatoid PDAC, long latency
93	Ptf1a-Cre;K-ras ^{G12D} ;p53 ^{lox/+} ;Smo ^{lox/lox}	mPanIN to PDAC progression	N/A	12 weeks	Deletion of Smo caused no additional phenotype
93	Ptf1a-Cre;K-ras ^{G12D} ;p53 ^{lox/+} ;Smo ^{lox/+}	mPanIN to PDAC progression	N/A	14 weeks	Improved median survival (17d) compared to Smo ^{lox/lox}
37	Ptf1a-Cre;K-ras ^{G12D} ;Rac1 ^{lox/lox}	Reduced mPanINs	No	>15 months	Extended survival, delayed mPanIN development
28	Ptf1a-Cre;K-ras ^{G12D} ;SMAD4 ^{lox/lox}	MCN to PDAC progression	Yes	8 months	Model of MCN-derived PDAC
30	Ptf1a-Cre;R26rtTA;tetO-LSL-K-ras ^{G12D} ;p53 ^{lox/+}	mPanIN to PDAC progression	Yes	4 months	Inducible PDAC model