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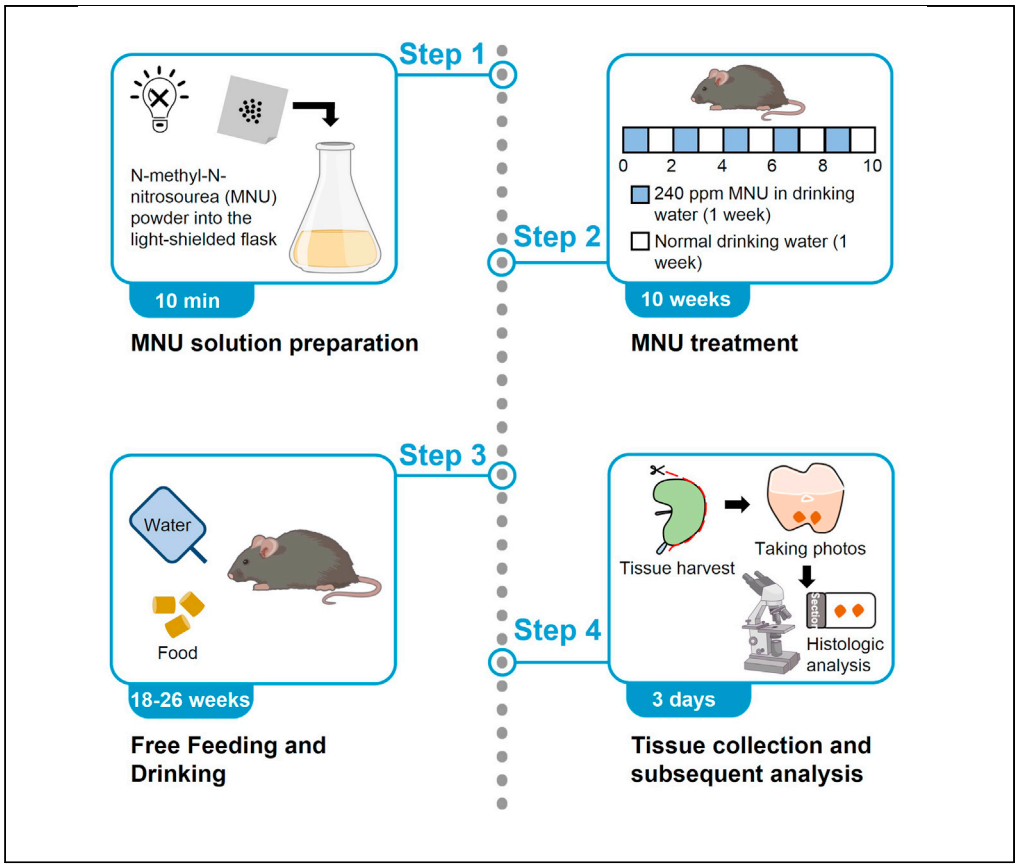
Protocol for chemically induced murine gastric tumor model



N-Methyl-N-nitrosourea, an N-nitroso compound converted from dietary nitrite by *Helicobacter pylori*, causes somatic mutations in epithelial cells and induces gastric premalignancy. Here, we describe a detailed protocol for induction of gastric tumor and analysis of tumor phenotypes in mice. This model can be widely used for studying the initiation and growth of gastric cancer.

Ke Li, Ao Wang, Huijuan Liu, Baojie Li

libj@sjtu.edu.cn (B.L.)
zlkeli@sjtu.edu.cn (K.L.)

Highlights

Preparation of N-methyl-N-nitrosourea (MNU) solution and *Helicobacter pylori*

Establishment of a murine gastric tumor model with MNU

Summary of development of gastric tumors and tumors in other organs

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Protocol for chemically induced murine gastric tumor model

Ke Li,[1,2,*] Ao Wang,[1] Huijuan Liu,[1] and Baojie Li[1,3,*]

[1]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai 200240, China

[2]Technical contact

[3]Lead contact

*Correspondence: libj@sjtu.edu.cn (B.L.), zlkeli@sjtu.edu.cn (K.L.)
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SUMMARY

N-Methyl-N-nitrosourea, an N-nitroso compound converted from dietary nitrite by *Helicobacter pylori*, causes somatic mutations in epithelial cells and induces gastric premalignancy. Here, we describe a detailed protocol for induction of gastric tumor and analysis of tumor phenotypes in mice. This model can be widely used for studying the initiation and growth of gastric cancer.

For complete details on the use and execution of this protocol, please refer to Li et al. (2021).

BEFORE YOU BEGIN

The protocol below describes the specific steps for wild-type and Tsc1-deficient C57BL/6 mice. However, we have also used the protocol for Mek1-deficient and Bmpr1a-deficient mouse lines. The animal experiments in this protocol have followed the recommendations of the National Research Council Guide for Care and Use of Laboratory Animals. All protocols have been approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University, China.

> ***Note:*** Gastric tumor models have been generated in other mouse strains including FVB, BALB/c, C3H/He and MSM (Table 1).

Prepare *Lgr5-GFP-Cre^{ERT}; Tsc1$^{f/f}$* mice

Timing: more than 6 months

1. Purchase the *Lgr5-GFP-Cre^{ERT}* mice and *Tsc1$^{f/f}$* mice from the Jackson Laboratories and breed them to the fertile age.
2. Cross *Lgr5-GFP-Cre^{ERT}* mice with *Tsc1$^{f/f}$* mice.
3. Genotype the offspring. Mouse tails are lysed in a lysis buffer containing Proteinase K (2%) and genomic DNA is extracted with isopropanol precipitation. We wash the DNA pellets with 70% ethanol and dry the pellet. The DNA is used for genotyping with PCR.

> ***Note:*** The first filial generation is *Lgr5-GFP-Cre^{ERT}; Tsc1$^{f/+}$* and we need cross these mice to generate *Lgr5-GFP-Cre^{ERT}; Tsc1$^{f/f}$* mice.

Table 1. Tumor formation induced by MNU in various organs of different mouse strains

Tumors of organ	Treatment	Strains of mouse	Concentration and administration[a]	Time of MNU treatment	Duration after MNU treatment	Incidence	Reference
Stomach	MNU	C57BL/6	240 ppm, d.w	5 weeks	22 weeks	60%	Li et al. (2021)
	MNU	C56BL/6, FVB	240 ppm, d.w	5 weeks	26 weeks	90%	Tomita et al. (2011)
	MNU	C57BL/6	120 ppm, d.w	5 weeks	40 weeks	80%	Yamamoto et al. (2000)
	MNU	C57BLKS	60 ppm, d.w	30 weeks	0 weeks	57%	Yoshizawa et al. (2009)
	H. pylori + MNU	C57BL/6, FVB	240 ppm, d.w	5 weeks	26 weeks	100%	Tomita et al. (2011)
	H. pylori + MNU	C57BL/6	200 ppm, d.w	5 weeks	40 weeks	85%	Lee et al. (2015)
	MNU	BALB/c	240 ppm, d.w	5 weeks	40 weeks	64%	Yamachika et al. (1998)
	MNU	BALB/c	120 ppm, d.w	10 weeks	30 weeks	54%	Yamachika et al. (1998)
	MNU	BALB/c	60 ppm, d.w	20 weeks	30 weeks	58%	Yamachika et al. (1998)
	MNU	C3H/He	120 ppm, d.w	30 weeks	12 weeks	81.5%	Tatematsu et al. (1993)
	MNU	C3H/He	60 ppm, d.w	30 weeks	12 weeks	60.7%	Tatematsu et al. (1993)
	MNU	C3H/He	30 ppm, d.w	30 weeks	12 weeks	63%	Tatematsu et al. (1993)
	MNU	MSM	0.03 mg/g. i.g	10 weeks	36 weeks	6.3%	Masui et al. (1997)
Intestine (duodenum)	MNU	C57BL/6	240 ppm, d.w	5 weeks	20 weeks	0%–6.3%	Ogawa et al. (2013)
	MNU	C57BL/6	50 mg/kg, i.p	1 time	20–30 weeks	14%	Qin et al. (2000)
	MNU	C57BL/6	50 mg/kg, i.p	1 time	30–40 weeks	56%	Qin et al. (2000)
Colon	*H. pylori* + MNU	C57BL/6J	240 ppm, d.w	5 weeks	70 weeks	85%	Ogawa et al. (2013)
Lung	MNU	C57BL/6	240 ppm, d.w	5 weeks	20 weeks	11%–25%	Ogawa et al. (2013)
	MNU	BALB/c	120 ppm, d.w	6 weeks	50 weeks	23%	Faustino-Rocha et al. (2015)
	MNU	A/J	50 mg/kg, i.p	4 weeks	32 weeks	N/A	Westcott et al. (2015)
Bladder	MNU	C3H/He	7.5 mg/mL, i.ves	1 time	4 weeks	28%	Soloway et al. (1983)
Mammary gland	MNU	BALB/c	5 mg/100g, i.p	3 doses 1 month	26 weeks	15%	Pazos et al. (1992)
Kidney	MNU	C57BL/6	240 ppm, d.w	5 weeks	20 weeks	5%–12%	Ogawa et al. (2013)
Liver	MNU	C57BL/6	240 ppm, d.w	5 weeks	20 weeks	18%–22%	Ogawa et al. (2013)

[a] d.w represents drinking water; i.g represents intragastric intubation; i.p represents intraperitoneal injection; i.ves represents intravesical injection.

Primers for genotyping	
Primer 8060	5′- CTGCTCTCTGCTCCCAGTCT -3′
Primer 8061	5′- ATACCCCATCCCTTTTGAGC -3′
Primer 9402	5′- CACCCCGGTGAACAGCTC -3′
Primer 4008	5′- GTCACGACCGTAGGAGAAGC -3′
Primer 4009	5′- GAATCAACCCCACAGAGCAT -3′

PCR reaction system for *Lgr5* knock-in allele	
Reagent	Amount
Primer 8060	0.6 μL
Primer 8061	0.8 μL
Primer 9402	0.4 μL
ddH_2O	3.7 μL
2× Taq Mix Buffer	6.5 μL
Sample DNA	1 μL
Total	13 μL

PCR reaction system for *Tsc1* floxed allele	
Reagent	Amount
Primer 4008	0.5 μL
Primer 4009	0.5 μL
ddH_2O	9.5 μL

(*Continued on next page*)

Continued

PCR reaction system for *Tsc1* floxed allele

Reagent	Amount
2× Taq Mix Buffer	12.5 μL
Sample DNA	2 μL
Total	25 μL

PCR cycling conditions for *Lgr5* knock-in allele

Steps	Temperature	Time	Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 s	35 cycles
Annealing	66°C	1 min	
Extension	72°C	30 s	
Final extension	72°C	2 min	1
Hold	4°C	forever	

PCR cycling conditions for *Tsc1* floxed allele

Steps	Temperature	Time	Cycles
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	30 s	35 cycles
Annealing	60°C	1 min	
Extension	72°C	1 min	
Final extension	72°C	2 min	1
Hold	4°C	forever	

Cre/Lox P system working

Timing: 3 days

4. Each male mouse received intraperitoneal injection of tamoxifen (TAM) (2 mg/20 g body weight) for 3 consecutive days at 2 months of age.

   Note: TAM can be dissolved in corn oil and the concentration should be low, due to possible damage to the stomach at high concentrations.

   Note: TAM should be injected two weeks before starting tumor induction.

   Optional: for H. pylori preparation, it can be grown on trypticase broth agar medium containing 5% defibrinated sheep blood with microaerobic atmosphere (3–5% O_2 and 10% CO_2) at 37°C. The bacteria are harvested after 48 hrs of growth and re-suspended in trypticase broth for subsequent use.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Proteinase K	Millipore	Cat #539480
Tamoxifen	Sigma	Cat #T5648
Corn oil	Aladdin	Cat #C116023
N-Methyl-N-nitrosourea (MNU)	Macklin	Cat #684-93-5
2× Taq Mix buffer	Abmgood	Cat #G013

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Ketamine	Sigma	Cat #K-002
Xylazine	Sigma	Cat #X1126
EDTA	Sangon Biotech	Cat #60-00-4
NaCl	Sangon Biotech	Cat #7647-14-5
Tris	Vetec	Cat #77-86-1
KH_2PO_4	BBI	Cat #7778-77-0
Na_2HPO_4■12H2O	Sangon Biotech	Cat #7782-85-6
KCl	Sangon Biotech	Cat #7447-40-7
SDS	BBI	Cat #151-21-3
Ethanol	Sinopharm	Cat #10009218
Isopropanol	Sinopharm	Cat #80109218
Dimethyl benzene	Lingfeng	Cat #1330-20-7
2,2,2-Tribromoethanol	Sigma	Cat #T48402
2-Methyl-2-butanol	Sigma	Cat #471712
HCl	Lingfeng	Cat #6747-01-0
Paraformaldehyde	Macklin	Cat #P804537
Paraplast High Melt	Leica	Cat #39601095
Tryptone	Sangon Biotech	Cat #A505250
Soytone	Sangon Biotech	Cat #A600214
Trypticase Soy Broth (TSB), Bottled Broth	Sigma	Cat #Z699195
U0126	Selleck	Cat #s1102
LDN-193189	Selleck	Cat #s2618
Experimental models: organisms/strains		
Mouse: *Lgr5-GFP-Cre^{ERT}* (C57BL/6, male, 2 month-old)	Dr. Hans Clever, Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences (KNAW), the Netherlands	N/A
Mouse: *Tsc1$^{f/f}$* (C57BL/6, male, 2 month-old)	The Jackson Laboratories	JAX: 005680
Organism: *Helicobacter pylori*	ATCC	Cat #49179
Oligonucleotides		
Primer 8060: 5′-CTGCTCTCTGCTCCCAGTCT-3′	This paper	N/A
Primer 8061: 5′-ATACCCCATCCCTTTTGAGC-3′	This paper	N/A
Primer 9402: 5′-CACCCCGGTGAACAGCTC-3′	This paper	N/A
Primer 4008: 5′-GTCACGACCGTAGGAGAAGC-3′	This paper	N/A
Primer 4009: 5′-GAATCAACCCCACAGAGCAT-3′	This paper	N/A
Other		
Aluminum foil	Aka	Cat #8011-O
8-Strip PCR tube	LABTIDE	Cat #P01-0803C
8-Strip flat cap	LABTIDE	Cat #P01-0803B
Mircrotubes (1.5 mL)	Axygen	Cat #MCT-150-C
General surgical scissor	RWD Life Science	Cat #S14001
General surgical tweezer	RWD Life Science	Cat #F11020
Disposable syringes (1mL)	KDL, Shanghai	Cat #KDL-1mL
Sterile gummed tape	3M	Cat #1322
Tissue Embedding Cassettes	Xiuwei Commerce	Cat #BMH-002
Sterile Cotton Swabs	Medicomp	Cat #4215352
Water bottles for mouse cages	Baoy, Beijing	Cat #By100

MATERIALS AND EQUIPMENT

Mice tail lysis buffer

Reagent	Final concentration	Amount
Tris-HCl (pH 8.0)	1 M	100 mL
SDS	10% (w/v)	40 mL

(*Continued on next page*)

Continued		
Reagent	Final concentration	Amount
EDTA (pH 8.0)	0.5 M	10 mL
NaCl	5 M	40 mL
Sterilized water	N/A	810 mL
Total	**N/A**	**1000 mL**

Sterilize, filter, and store at 24°C–25°C. Before use, add 2% (v/v) Proteinase K.

Note: Mouse tail lysis buffer may suffer from salting out at 4°C. However, it can be used after brief heating and re-dissolving.

PBS buffer		
Reagent	Final concentration	Amount
NaCl	137 mM	8 g
KH_2PO_4	1.47 mM	0.24 g
$Na_2HPO_4 \cdot 12H_2O$	10 mM	3.58 g
KCl	2.7 mM	0.2 g
ddH_2O	N/A	1000 mL
Total	**N/A**	**1000 mL**

Sterilized by autoclaving and store at 24°C–25°C.

Trypticase broth/Agar		
Reagent	Final concentration	Amount
Tryptone	N/A	15 g
Soytone	N/A	5 g
NaCl	86 mM	5 g
Dextrose	14 mM	2.5 g
Agar	N/A	15 g
ddH_2O	N/A	1000 mL
Total	**N/A**	**1000 mL**

Sterilize and store at 4°C. For trypticase broth, omit agar.

Alternatives: Commercial trypticase broth medium can be purchased (see key resources table).

Other materials	
Name	Reagents
Tamoxifen	10 mg/mL in corn oil. Mix one night and store at 4°C
Proteinase K	100 mg dissolved in 5 mL sterilized water
MNU	Dissolved to 240 mg/L in sterilized drinking water
Avertin	5 g 2,2,2-Tribromoethanol dissolved in 3.1 mL 2-Methyl-2-butanol for storage at 4°C. Diluted 40 times for administration
PFA	4 g paraformaldehyde dissolved in 100 mL sterilized water

STEP-BY-STEP METHOD DETAILS

N-methyl-N-nitrosourea (MNU), a carcinogenic agent, generates somatic mutations in epithelial cells of the stomach and induces tumor formation. In this article, we show that the standard tumorigenesis protocol using MNU (240 ppm) can generate gastric tumors at high incidence in the antrum.

Note: Although MNU can be used to induce intestine and colon tumors, the ways of drug administration are quite different. Mice are usually injected with MNU by intraperitoneal

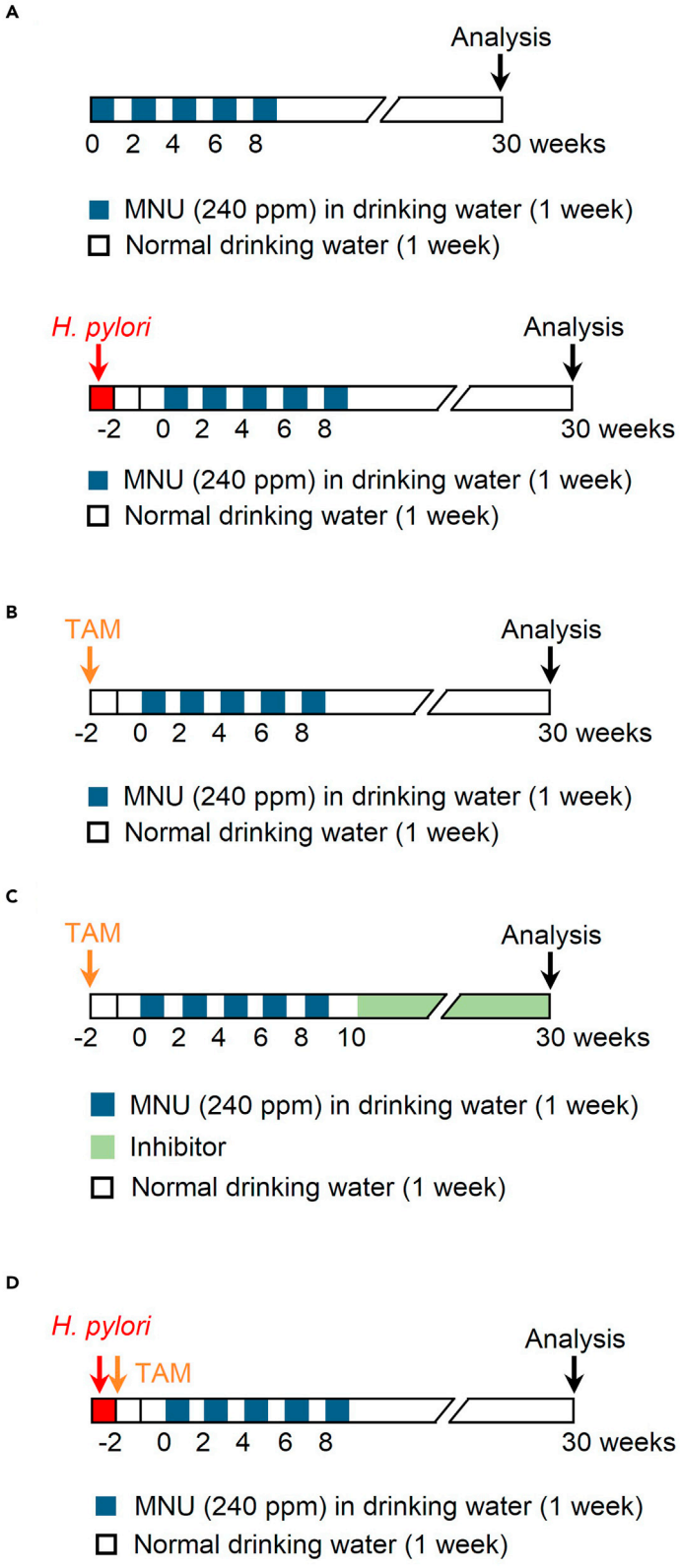

A
Analysis
0 2 4 6 8
30 weeks
MNU (240 ppm) in drinking water (1 week)
Normal drinking water (1 week)
H. pylori
Analysis
-2 0 2 4 6 8
30 weeks
MNU (240 ppm) in drinking water (1 week)
Normal drinking water (1 week)
B
TAM
Analysis
-2 0 2 4 6 8
30 weeks
MNU (240 ppm) in drinking water (1 week)
Normal drinking water (1 week)
C
TAM
Analysis
-2 0 2 4 6 8 10
30 weeks
MNU (240 ppm) in drinking water (1 week)
Inhibitor
Normal drinking water (1 week)
D
H. pylori
TAM
Analysis
-2 0 2 4 6 8
30 weeks
MNU (240 ppm) in drinking water (1 week)
Normal drinking water (1 week)

Figure 1. Induction of gastric tumor formation in normal and genetically modified mice
(A) A time line for MNU treatment with or without *H. pylori* infection in wild-type mice. Blue square represents 1 week duration of MNU treatment and red square represents *H. pylori* infection.
(B) A schedule for MNU treatment in TAM-induced genetically modified mice. Mice were administrated with TAM through intraperitoneal injection 3 times before MNU treatment.
(C) A schedule for inhibitor treatment in the MNU model. Green line represents the duration of inhibitor treatment.
(D) A scheme for MNU treatment with H. pylori infection in TAM-induced genetically modified mice.

administration for intestine tumor induction and by intrarectal administration for colon tumor induction.

MNU solution preparation

Timing: 10 min

This step allows you to dissolve MNU in drinking water.

1. Use a light-shielded bottle or cover a transparent one with appropriate size of aluminum foil.
2. Weigh 0.012 g MNU powder on a precise analytical balance under a light-shield condition.
3. Place the MNU into the bottle prepared in step 1.
4. Dissolve MNU in 50 mL sterile drinking water.

⚠ CRITICAL: Due to instability of MNU, we suggest preparing small amounts of the solution (50 ml) and change it periodically.

Note: MNU is a toxic carcinogen and photodecomposition occurs easily. Be very careful when handling it and use appropriated personal protective equipment.

Note: The concentration of MNU in drinking water is 240 ppm. It is known that more time is needed for lower concentrations of MNU to induce gastric tumors (Table 1).

MNU treatment

Timing: 10 weeks

Optional: To mimic the microenvironment of human stomach, mice can be infected with *H. pylori* in 0.2 ml trypticase broth by oral gavage 3 times per week, 2 weeks before MNU administration. The total dose of *H. pylori* is around 100 million colony-forming units per mouse (Figure 1A). The dose can be quantified by counting the bacteria on slides under microscope or routine culture plate counting method. *H. pylori* in the gastric gland can be detected by Warthin–Starry staining.

Tumorigenesis in murine stomach relies on oncogenic cues. Here, mice are exposed to MNU, a carcinogen generated by *H. pylori*, in the chemical-induced model (Figures 1A–1D).

Note: We recommend using mice at 2–3 months of age. We keep 4 mice in one cage.

5. Place the light-shielded water bottle containing MNU on the cage so the mice have free access.
6. Two days later, replace the MNU solution with newly prepared solution.

Note: Repeat the steps 1–4 in the end of step 6. Due to instability of MNU, we suggest the frequency of change is thrice per week or every other day to keep the potency of the agent.

7. After one week of MNU treatment, take out the light-shielded bottle and change it for the normal transparent bottle containing just drinking water.
8. One week later, the transparent bottle should be changed for the light-shielded bottle containing MNU solution.

⚠ CRITICAL: Steps 5–8 comprise one cycle (2 weeks). Repeat the treatment for 5 cycles in wild-type or TAM-induced genetically modified mice.

9. Repeat step 5 to step 8 for 4 more cycles (Figure 1A).

Free feeding and drinking

Timing: approximately 22 weeks

10. Mice are housed in a pathogen-free facility with free access to food and water waiting for the development of gastric tumors, for approximately 22 weeks.

Note: The feeding period after treatment with MNU is flexible and the mice can be sacrificed from weeks 18–26. In addition, the feeding time with low concentration MNU (60 or 120 ppm) should increase to 32–40 weeks.

Optional: The feeding time is an appropriate period for other treatments. Mice can be administered with inhibitors, such as U0126 and LDN-193189, MEK1 and BMPR1A inhibitors respectively, or other drugs via intraperitoneal injection every other day (Figure 1C).

Tumor tissue collection and subsequent process

Timing: 3 days

Collect the stomach tissues, take photos of tumors and perform H&E or immuno-staining.

11. Anesthetize the mice using 40 mg/mL Avertin (240 mg/kg body weight) by intraperitoneal injection.

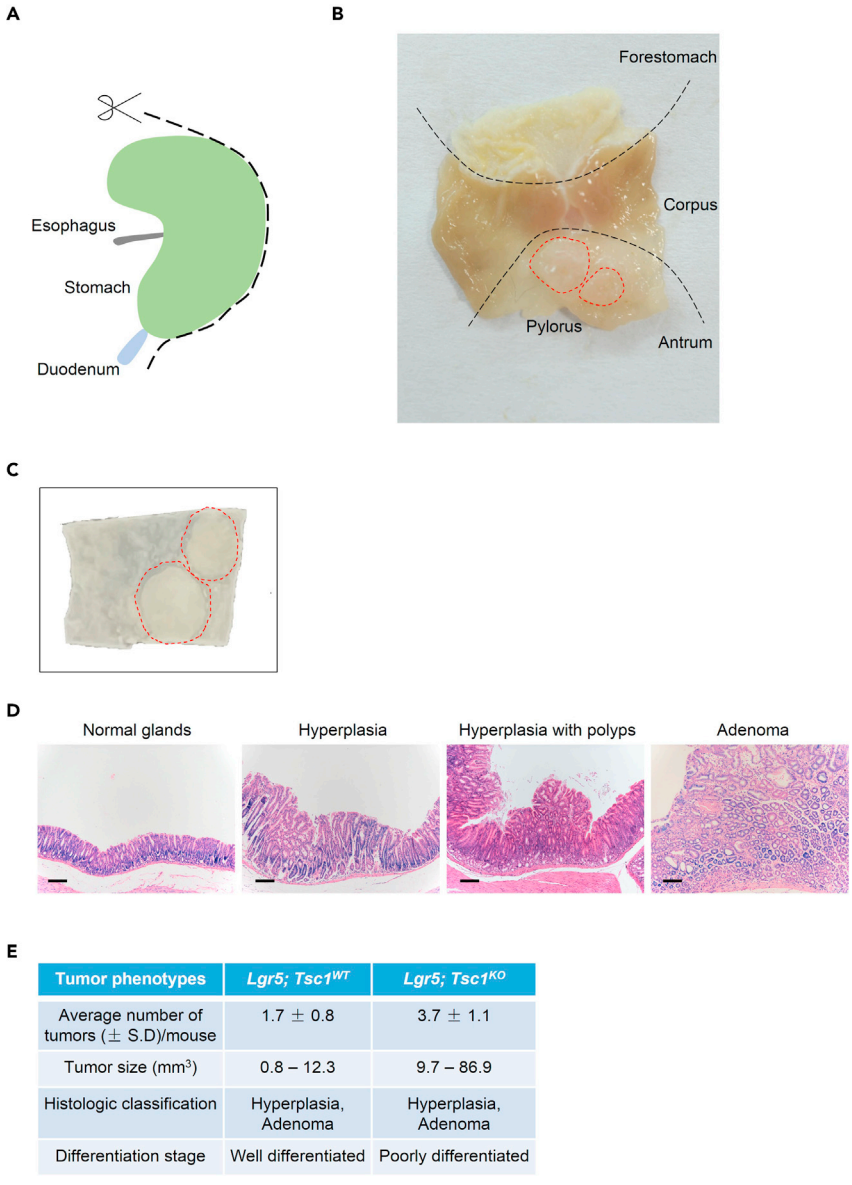
Alternatives: In addition to Avertin, mice can be anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) via intraperitoneal injection.

12. Lay down the animal and fix it on the surgical platform, remove the ribs and carefully expose the heart avoiding the rupture of other tissue, especially the major blood-vessels near the heart.
13. Perfuse sterilized PBS to flush out blood from the heart after removing the auricle (Methods video S1).

⚠ CRITICAL: During perfusion, inject the PBS slowly at a constant speed. The color of lungs and livers should become light, indicating the loss of blood after a successful perfusion.

14. After perfusion, carefully harvest the stomach and then open the organ along the great curve, wash the tissue with cold PBS three times to remove the food debris (Figure 2A).
15. Unfold the stomach on a white platform or paper to expose the interior of forestomach, corpus, and antrum, and take photos (Figure 2B). Next, quantify the number of tumors in each animal and calculate their volume using a caliper.

Note: The number of tumors is around 2 and the size is from 0.8 to 12 mm^3 in wild-type mice. In some genetically modified mice, the size is up to 80 mm^3 (Figure 2E).



E

Tumor phenotypes	*Lgr5; Tsc1^{WT}*	*Lgr5; Tsc1^{KO}*
Average number of tumors (± S.D)/mouse	1.7 ± 0.8	3.7 ± 1.1
Tumor size (mm^3)	0.8 – 12.3	9.7 – 86.9
Histologic classification	Hyperplasia, Adenoma	Hyperplasia, Adenoma
Differentiation stage	Well differentiated	Poorly differentiated

Figure 2. Tumor tissue collection and measurement

(A) A diagram for stomach harvest. Remove esophagus and duodenum and then cut along the dotted line in the great curve to expose the interior of the stomach.

(B) A photo of tumor formation in the antrum near pylorus. Red circles indicate the contour of the tumors.

(C) A photo of gastric tumor after fixing. Red circles indicate the contour of the tumors.

(D) H&E staining showed the features of normal gastric gland, hyperplasia (polyp) and adenoma in MNU models. Scale bar: 100 μm.

(E) Tumor numbers, average size, and histologic grades in mice induced by MNU. Data are represented as mean ± SD, n = 7 per group, this figure has been modified from Li et al. (2021).

16. Cut the tumor tissues into several pieces. One part is for histopathological analysis and other parts are frozen for future RNA or protein extraction.
17. Place the tissue into 4% PFA solution to fix it one night and transfer to 70% ethanol for histological analysis.

Pause point: Tissues can be kept in 70% ethanol at 4°C for a short period of time (l week maximal) before proceeding to the next step.

18. Perform the routine dehydrating and paraffin embedding procedures for H&E staining.
    a. Transfer tissues from PFA solution to 70% ethanol for 1 h.
    b. Dehydrate the tissues in increasing concentrations of ethanol, 80%, 90%, 95%, and 100% for 1 h each.
    c. Immerse the tissues in dimethyl benzene (100%) for derosination and transparency enhancement for 1 h and then wax them for 3 h. The paraffin should be changed once every hour.
    d. Embed the tissue in paraffin blocks and cut them into 4–5 μm sections for H&E staining or other histological staining.

Optional: Between dehydration and dimethyl benzene treatment, tissues can be transferred to a mix of absolute ethanol and dimethyl benzene for 1 hour, as an intermediate stage.

Note: The histologic classification of tumors can be defined by H&E staining. The sign of hyperplasia is the dysplasia of gastric epithelium with elongation of gland units, and the advanced tumor with glandular and cellular distortion is considered to be adenoma (Figure 2D).

EXPECTED OUTCOMES

Tumors will mostly form and grow in the pylorus and antrum of stomach (Figure 2B), sometimes in the corpus. Furthermore, a swelling/hyperplasia of the epithelia can be found in the non-tumor area. Due to the heterogeneity of the size and number of tumors in the animals, we usually use more than 10 mice. In case of drug treatment or use of genetically modified mice, the time for gastric tumor development may change.

The protocol of MNU with high concentration (short treatment time) is commonly used; low concentration may require long time of drug treatment to induce gastric tumor formation (Yamachika et al., 1998). We summarized the incidence of tumors in the stomach and other organs in various strains (Table 1).

QUANTIFICATION AND STATISTICAL ANALYSIS

The numbers of tumors in the stomach are counted visually. For the volume, the length, width, and height of each tumor/polyp are measured by vernier caliper, multiplying length by width and height to get the tumor volume.

LIMITATIONS

MNU-induced murine tumor model can be used to study the initiation and development of gastric tumors. It mimics human gastric cancer in several ways: somatic DNA mutations, microbial infection, and similarity between MNU and diet-contained nitrate. Furthermore, MNU can induce hyperplasia (polyps) and adenoma, similar to human gastric tumors. However, there are limitations for this model. The risk factors are much complex in humans and MNU only mimics diet-contained nitrate. Due to the long time needed for the development of tumors, mice may die prematurely especially in mice with genetic modifications. Moreover, there is no obvious sign to assess tumor formation before sacrificing the mice.

TROUBLESHOOTING

Problem

The amount of MNU powder is too little to weigh (step 2).

Potential solution

We recommend the smallest measuring scoop or hand-made small scoop using a plastic straw, which, together with MNU, can be directly dropped into the light-shielded bottle.

Problem
Mouse death during the treatment (step 10).

Potential solution
MNU may affect other organs and tissues, such as lung, kidney and liver, causing injury and/or carcinogenesis. The incidence of lung, kidney and liver hyperplasia is 11, 5 and 18 percent respectively (detailed in Table 1). Low concentrations may reduce mouse mortality. For example, the concentration of MNU can be 120 ppm and the number of cycle is still 5.

Problem
Tumor in the duodenum (step 15).

Potential solution
The main region of gastric tumor initiation and development is antrum and corpus. However, sometimes the tumors growing in the pylorus may extend into the duodenum, with an incidence of 6%. When harvesting the tissues, you need carefully remove the gut and keep a part of duodenum with tumors, open it and take photos.

Problem
No tumor observed in the stomach in some animals (step 15).

Potential solution
In MNU-induced models, the incidence of tumors exhibits some variability. However, the incidence of dysplasia in wild-type mice is 60%–90%, which may increase to 100% with H. pylori infection (Sethi et al., 2020; Tomita et al., 2011). Alternatively, some small polyps can be observed and measured under dissecting microscope, which are also suitable for histopathological analysis (Figure 2D).

Problem
Failure of perfusion (step 13).

Potential solution
At the beginning, you should immediately and carefully open the thoracic cavity after anesthesia to avoid blood coagulation or hemorrhage. You need correctly insert the syringe needle into the left ventricle and break the right auricle to establish the perfusion loop. Moreover, the perfusion solution should be injected at a slow and constant speed to prevent vessel bursting.

RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Baojie Li (libj@sjtu.edu.cn)

Materials availability
This study did not generate new unique reagents.

Data and code availability
This study did not generate/analyze datasets.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2021.100814.

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AUTHOR CONTRIBUTIONS

B.L. designed the research; K.L. and A.W. wrote the original draft; B.L. and H.L. reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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