Abstract ID: #3008

Poster Board Number: A109

N-methyl-*N*-nitrosourea–induced forestomach neoplastic lesions are formed in rasH2 mice at 13 weeks after a single intra-abdominal injection

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Background and Objective

CByB6F1-Tg(HRAS)2Jic mice (rasH2) were developed as an alternative model to the conventional 2-year bioassay used for carcinogenicity testing.

The mice have been produced in three breeding facilities, CLEA Japan, Inc. (Fujinomiya, Shizuoka, Japan) and Taconic Bioscience (Germantown, NY, USA and Tornbjerg, DK).

To maintain phenotype fidelity and carcinogenic susceptibility in rasH2 mice, both colonies have been renewed within 10 generations. Insertion of the c-Ha-Ras gene is verified in all mice through PCR method. After colony renewal, we systematically conducted a 26-week carcinogenicity study utilizing *N*-methyl-*N*-nitrosourea (MNU) to ensure quality assurance.

This protocol includes i) comprehensive volume monitoring to evaluate sensitivity to MNU across all organs to ensure comparability, and ii) simplified volume monitoring to verify consistent forestomach sensitivity.

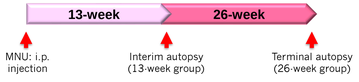
Our background and published data revealed that forestomach squamous cell papilloma or carcinoma was detected in over 80% of cases within 12 to 13 weeks following MNU administration. Consequently, we hypothesized that a simplified monitoring study could be effectively completed within a 13-week period.

In the present study, we investigated whether carcinogenic susceptibility in the forestomach remained consistently detectable when the observation period was set at 13 weeks by comparing to that at 26 weeks following MNU administration.

Materials and Methods

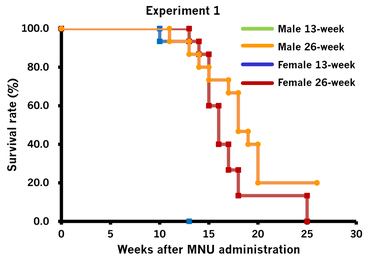
- **Animal:** Both sexes of rasH2 mice (CLEA Japan, Inc.), 5- or 6-week-old at the receipt
- **Test chemical:** *N*-methyl-*N*-nitrosourea (MNU) was purchased from Medchemexpress.
- **Administration of test chemical:** Mice aged 7-week-old were treated with 75 mg/kg of MNU solution by a single i.p. injection.
- **Observation period:** necropsies were performed either at 13th or 26th week after the MNU administration.
- **Observation and Examination Items:** Survival rate, body weight, grosspathological and histopathological examination.
- **Judgement criteria:** With tumor incidence in forestomach exceeding 80% indicating sustained carcinogenic susceptibility.

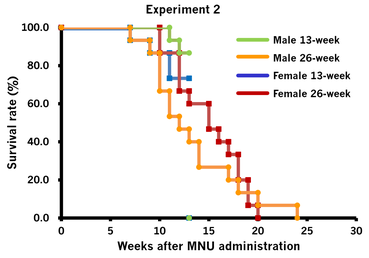
Study design



Experimental No.	Sex	Dose of MNU (mg/kg)	Observation period	No. of animals
1	Male/Female	75	13	15
			26	15
2	Male/Female	75	13	15
			26	15
3	Male/Female	75	13	10

Two comparative studies of 13- and 26- week group (Experiments 1 and 2) and one 13-week group validation study (Experiment 3) were conducted.





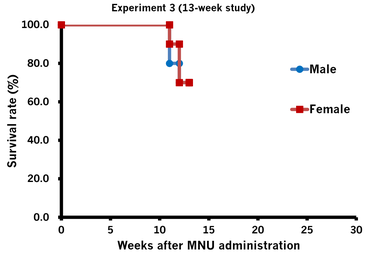


Figure 1. Survival curves

Dead or moribund animals were observed from 8 weeks after MNU administration, with their numbers gradually increased.

Survival curves exhibited a consistent trend across both experiment 1 and 2.

In the 26-week group, survival rates were 20.0 or 0.0% for males and females in experiment 1, and 0.0% for both sexes in experiment 2.

In experiment 3, the survival rate at week-13 after MNU administration was 70.0% for both male and female.

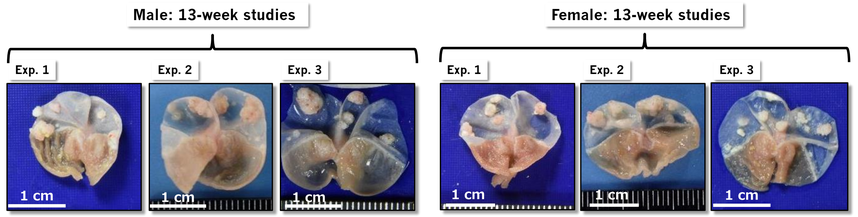
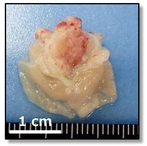


Figure 2. Representative macroscopic observation of the forestomach

In the 13-week necropsy animals across 3 experiments, multiple papillary nodules measuring approximately range 1 to 5 mm in diameter were sporadically observed in the forestomach.



Forestomach nodules were observed in all mice of both sexes in both the 13-week or 26-week groups. In some cases, large nodules exhibited hemorrhage with penetration extending to the serosal surface.

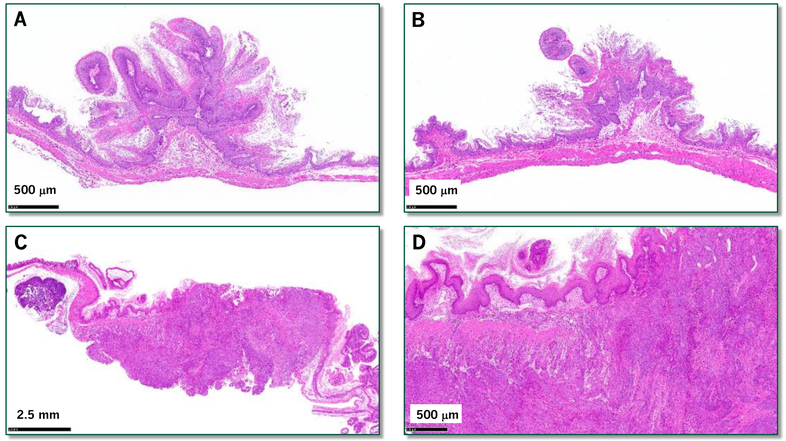


Figure 3. Representative histopathological images of the forestomach

Typical squamous cell papilloma observed in male or female of rasH2 mice in 13-week study (A and B).

Squamous cell carcinoma was observed in some cases in either 13-week or 26-week group (C).

Higher magnification of photo C shows the penetration of submucosa, muscle layer, and serosa (D).

Main cause of death in the moribund or found dead animals was thymic malignant lymphoma, a frequent histopathological finding in MNU-treated rasH2 mice.

Table 1. Neoplastic lesion of the forestomach over the three studies

Organs	Sex	MNU (mg/kg)	Exp. No.	Observation Period (week)	No. of animals	Papilloma, squamous cell	Carcinoma, squamous cell	Incidence (%)
Forestomach	Male	75	Exp. 1	13	15	15 (100.0)	0 (0.0)	15 (100.0)
				26	15	15 (100.0)	0 (0.0)	15 (100.0)
			Exp. 2	13	15	15 (100.0)	1 (6.7)	15 (100.0)
				26	15	15 (100.0)	1(6.7)	15 (100.0)
			Exp. 3	13	10	10 (100.0)	0 (0.0)	10 (100.0)
	Female	75	Exp. 1	13	15	14 (93.3)	1 (6.7)	15 (100.0)
				26	15	14 (93.3)	0 (0.0)	14 (93.3)
			Exp. 2	13	15	14 (93.3)	2 (13.3)	15 (100.0)
				26	15	14 (93.3)	0 (0.0)	14 (93.3)
			Exp. 3	13	10	10 (100.0)	0 (0.0)	10 (100.0)

Table 2. Summary of histopathological examination of neoplastic lesion in the forestomach from 26-week carcinogenicity studies conducted over the past 5 years (2019-2024)

Supplier	Sex	MNU (mg/kg)	No. of animals	Papilloma, squamous cell	Carcinoma, squamous cell	Papilloma or carcinoma	Incidence (%)
CLEA	Male	75	65	60	7	64/65	98.5
	Female	75	65	63	2	65/65	100.0
Taconic	Male	75	85	81	5	84/85	98.8
	Female	75	85	82	2	84/85	98.8

Histopathological examination exhibited that incidence of neoplastic lesion of the forestomach in the 13-week observation period across three studies was 100.0% in both sexes of rasH2 mice.

Historical control data from CIEM 26-week carcinogenicity studies involving Taconic and CLEA Japan mice exhibited that incidence of neoplastic lesions in the forestomach was exceeding 98.5%.

Conclusions

Based on these findings, the forestomach tumor incidence after 13-week MNU administration is deemed to match the established criterion (exceeding 80.0% forestomach tumor incidence) in alignment with the 26-week simplified volume monitoring carcinogenicity study.

In the upcoming simplified volume-monitoring carcinogenicity studies, the experimental period will be shortened from 26 weeks to 13 weeks.

COI

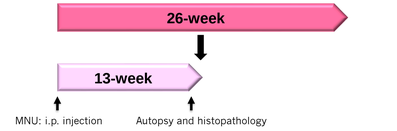
The authors have no financial conflicts of interest to disclose concerning the presentation

Future phenotypic monitoring system

Full volume-monitoring study =No change (26 weeks study)

- Histopathological analysis: whole body organs and tissue

Simple-monitoring study =Study period shortened to 13 weeks



- Histopathological analysis: forestomach squamous cell tumor (the organ most sensitive to MNU)