Establishment of the drug evaluation system using PDX-bearing BRJ mice

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Objective

Patient-derived xenograft (PDX) has recently been recognized as a highly predictive model for antitumor drug discovery. To establish PDX models, NOG mice are commonly used as an animal recipient in Japan. NOG mice have a genetic mutation in the *prkdc* gene related to DNA damage repair and are highly sensitive to DNA damage. In this study, we examined to establish a PDX model using mice without *prkdc* gene mutation.

We chose BALB/c Rag-2$^{-/-}$ JAK3$^{-/-}$ (BRJ) mice (Ono A. *et al.,* J. Biomed. Biotechnol., 2011) as a PDX recipient because BRJ mice, which do not have the *prkdc* gene mutation, show severe immunodeficiency comparable to NOG mice. To obtain tumor growth data in BRJ mice, PDXs were transplanted subcutaneously into female and male BRJ mice (gastric cancer: 3, lung cancer: 4, colon cancer 3, 10 PDXs in total). In addition, to evaluate tolerance to DNA damage in BRJ mice, NOG and BRJ mice were treated with Dox. Furthermore, to evaluate antitumor efficacy in BRJ mice, lung cancer PDX-bearing BRJ mice were treated with approved antitumor drugs [CDDP, DTX, CDDP+DTX or ALK inhibitor (Lorlatinib or Brigatinib)].

Summary in Japanese

臨床予測性の高いがんモデルとして，患者由来腫瘍移植モデル（PDX）が近年注目されている．本邦では，NOGマウスがPDXモデル作製に広く用いられている．NOGマウスはDNA損傷の修復に関わる*prkdc*遺伝子に変異を持つため，DNA損傷に高い感受性を持つ．DNA損傷を引き起こす物質はがん治療のためにしばしば用いられるため，我々は*prkdc*遺伝子変異を持たないマウスを用いたPDXモデルを検討し，NOGマウスと比較した．

BALB/c Rag-2$^{-/-}$ JAK3$^{-/-}$（BRJ）マウスはNOGマウスに匹敵する重度免疫不全マウスであり，*prkdc*遺伝子の変異を持たない．そのため，BRJマウスをPDXのレシピエントとして選択した．BRJマウスでのPDX生着を検討するため，PDXをNOGマウスとBRJマウスに移植した（肺がん4種，大腸がん3種，胃がん3種，合計10株）．その結果，BRJマウスでもNOGマウスと同様のPDX増殖が見られた．

次に，DNA損傷を引き起こす薬剤に対するBRJマウスの忍容性を評価した．肺がんPDXを移植したBRJマウス及びNOGマウスにDoxを投与した（PDX名称：LU-016-LSIM, EML4-ALK融合遺伝子陽性肺腺がん）．その結果，BRJマウスではDox 6 mg/kg投与により体重減少が見られたが，抗腫瘍効果を評価することができた．一方，NOGマウスでは体重減少や死亡により薬効評価が困難であった．また，生存期間についてはBRJマウスはNOGマウスよりも長期に生存することができたため，BRJマウスはDNA損傷に対しNOGマウスよりも耐性を示すことが示唆された．

また，PDXを移植したBRJマウスを用いた薬効評価系も検討した．LU-016-LSIMを移植したBRJマウスに既存の抗腫瘍薬を投与した［CDDP, DTX, CDDP+DTXまたはALK阻害剤（LorlatinibまたはBrigatinib）］．その結果，CDDP単剤では有意な抗腫瘍効果を示さなかったが，DTX単剤は抗腫瘍効果を示し，CDDP+DTXにより抗腫瘍効果は増強された．また，ALK阻害剤はどちらも抗腫瘍効果を示した．

本研究によりBRJマウスを用いたPDXモデルの薬効評価系が確立された．本モデルはがん治薬のさらなる発展に貢献することが期待される．

Materials and Methods

【PDX】

PDX tumors obtained from National Cancer Center Research Institute were propageted in NOG mice and stored in a liquid nitrogen storage tank.

【Animal experiment】

Four to six weeks old female and male BRJ mice (BALB/c Rag-2$^{-/-}$ JAK3$^{-/-}$) obtained from Kumamoto University. Six weeks old female NOG mice (NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/ShiJic) obtained from Central Institute for Experimental Animals.

PDX tumor pieces were transplanted subcutaneously under isoflurane inhalation anesthesia.

Tumor diameter was measured using caliper and the volume was calculated by the following equation.

Estimated tumor volume (mm^3) =
1/2 × long diameter (mm) × short diameter (mm) × short diameter (mm)

To evaluate antitumor efficacy, PDX-bearing animals were assigned homogeneously to each test group by the "stratified randomization method" on the basis of the tumor volume.

【Reagent】

Doxorubicin (Dox, ADRIACIN Injection, Sandoz K.K.), Cisplatin (CDDP, Randa Inj., Nippon Kayaku Co., Ltd.) and Docetaxel (DTX, ONETAXOTERE I.V.Infusion, Sanofi K.K.) diluted by saline or undiluted were administrated intravenously once a week.

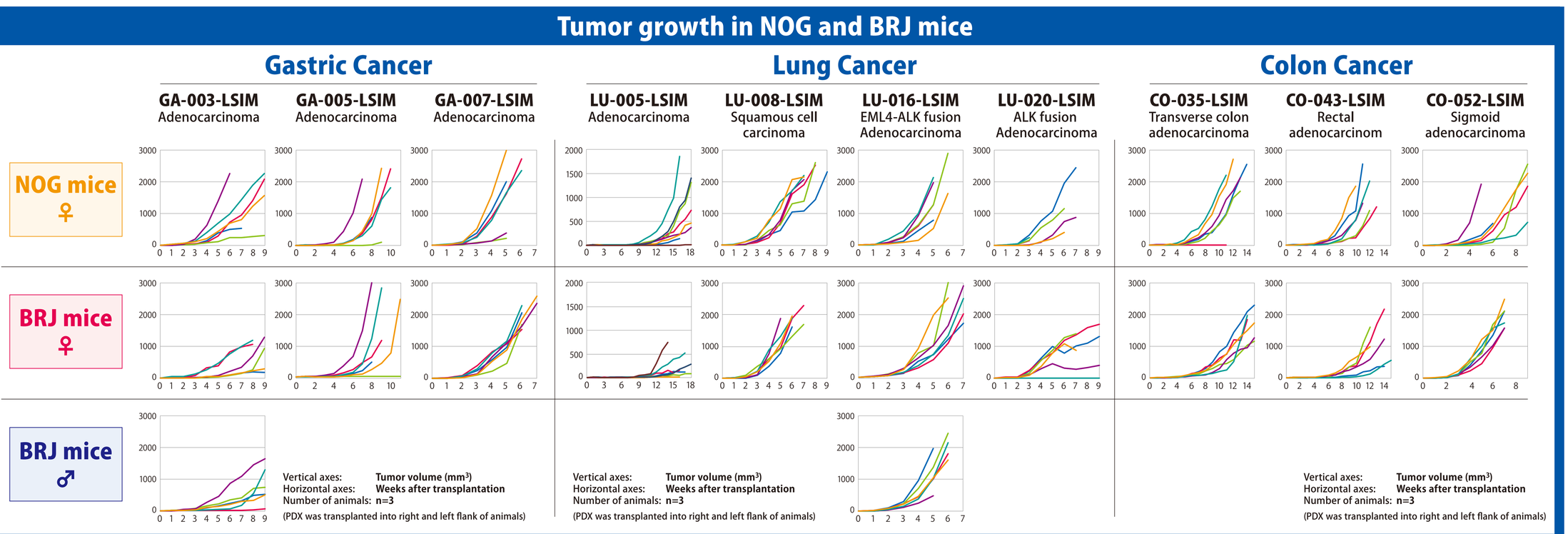
Lorlatinib (LORBRENA Tablets, Pfizer Japan Inc.) and Brigatinib (ALUNBRIG Tablets, Takeda Pharmaceutical Company Ltd.) were suspended in 0.5w/v% Methyl cellulose 400 solution and were administrated orally once a day.

【HE stain and immunohistochemistry (IHC)】

Paraffin-embedded blocks were created from tumor immersed in 10% formalin neutral buffer solution. Then, HE staining and IHC were performed. ALK (D5F3) XP Rabbit mAb (#3633, Cell Signaling Technology) was used for IHC. For ALK positive and negative control, tissue microarray slide of lung caner PDXs was stained by the same method.

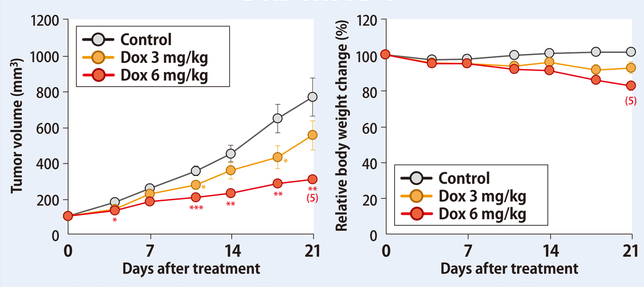
Results

Tumor growth in NOG and BRJ mice



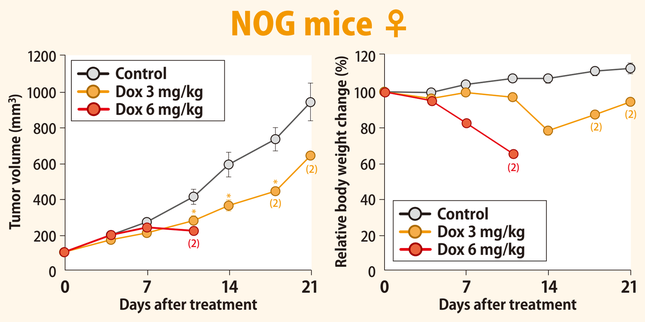
Comparison of tolerance to Dox administration in PDX-bearing BRJ and NOG mice (Lung cancer PDX: LU-016-LSIM)

BRJ mice ♂

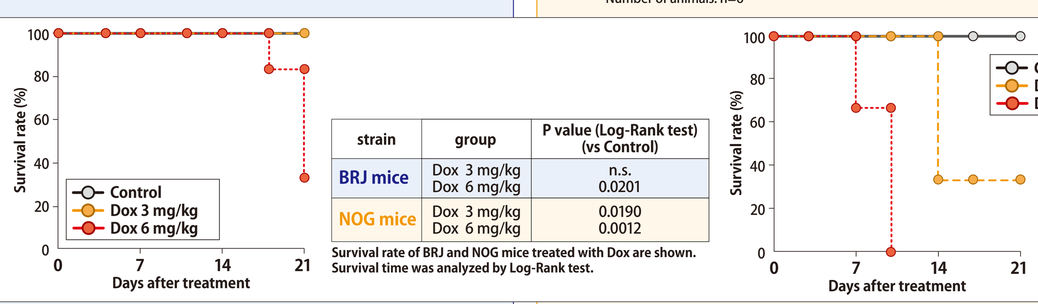


The day of group allocation was defined as Day 0 and BRJ mice were 14 weeks old.
Some animals were euthanized on Day 18 and Day 21 because of humane endpoint (body weight loss equal or more than 20% compared with that on Day0), but it was able to evaluate antitumor effect of Dox in BRJ mice.
Humane endpoint: One animal on Day 18 and four animals on Day 21 in the Dox 6 mg/kg group
*:p<0.05, **:p<0.01, ***:p<0.001, Dunnett's test (vs Control)
mean±SE
Number of animals: n=6

NOG mice ♀



The day of group allocation was defined as Day 0 and NOG mice were 10 weeks old.
It was difficult to evaluate antitumor effect of Dox because severe weight loss or death.
Equal or more than 20% body weight loss compared with that on Day 0 (humane endpoint):
Four animals on Day 14 in the Dox 3 mg/kg group. Each two animals on Day 7 and Day 10 in the Dox 6 mg/kg group.
Death: Two animals on Day 10 in the Dox 6 mg/kg group.
*:p<0.05, Student's t test (vs Control)
mean±SE
Number of animals: n=6

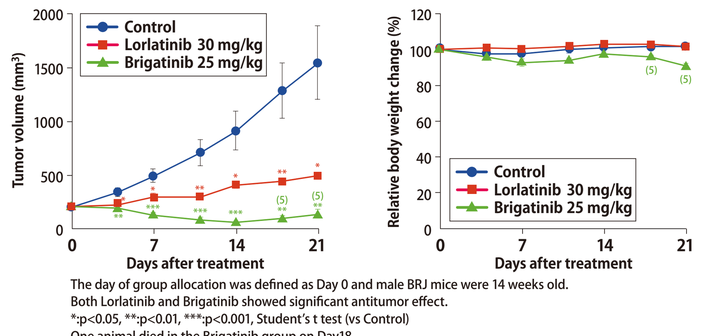


strain	group	P value (Log-Rank test) (vs Control)
BRJ mice	Dox 3 mg/kg	n.s.
	Dox 6 mg/kg	0.0201
NOG mice	Dox 3 mg/kg	0.0190
	Dox 6 mg/kg	0.0012

Survival rate of BRJ and NOG mice treated with Dox are shown.
Survival time was analyzed by Log-Rank test.

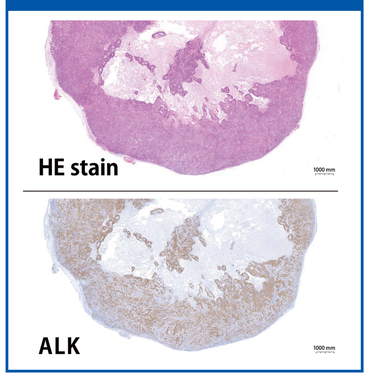
Drug evaluation in PDX-bearing BRJ mice (Lung cancer PDX: LU-016-LSIM)

The day of group allocation was defined as Day 0 and male BRJ mice were 14 weeks old.
DTX alone showed significant antitumor effect.
CDDP alone did not show antitumor effect, but combination enhanced antitumor efficacy.
*:p<0.05, Student's t test (vs Control), ##:p<0.01, Student's t test (vs DTX)

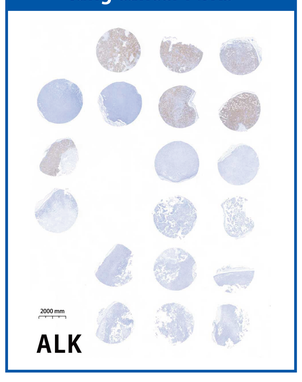


The day of group allocation was defined as Day 0 and male BRJ mice were 14 weeks old.
Both Lorlatinib and Brigatinib showed significant antitumor effect.
*:p<0.05, **:p<0.01, ***:p<0.001, Student's t test (vs Control)
One animal died in the Brigatinib group on Day18.

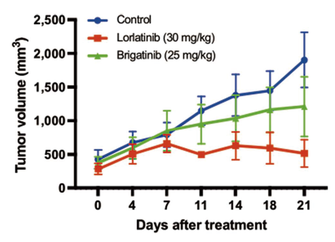
LU-016-LSIM



Tissue microarray of lung cancer PDXs



Supplemental figure and table



PDX: LC-002 TG4				
Drug	N	Day 21		
		%Gr	P	BW (%)
Control	4	-	-	-4.9
Lorlatinib	4	15.6	0.7625	0.2
Brigatinib	4	56.6	0.1318	6.7

Antitumor effect of ALK inhibitors in LU-016-LSIM-bearing NOG mice
H. Jo et al. Mol. Cancer Ther., 2022, Feb;21:359-370.
Comparative Study on the Efficacy and Exposure of Molecular Target Agents in Non–small Cell Lung Cancer PDX Models with Driver Genetic Alterations

Conclusion

In this study, we examined the drug evaluation system using PDX-bearing BRJ mice.

BRJ mice are reported to have severe immunodeficiency and high engraftment rate of human hematopoietic cells (Ono A. *et al.,* J. Biomed. Biotechnol., 2011), suggesting that a PDX model is able to be established using BRJ mice. PDXs of colon, gastric, and lung cancer grew in BRJ mice similarly or slightly slower than in NOG mice. PDXs used in this study were established using NOG mice; therefore, it was suggested these PDXs were adapted to grow in NOG mice. Accordingly, there is no problem with tumor growth in BRJ mice, and it is possible to use these mice as a PDX recipient.

DNA damage inducers are often used for cancer treatment, but many immunodeficient mice used for cancer research such as SCID, NOG, and NSG mice are not suited for experiments using DNA damage inducers. The reason is that these mice have the *prkdc* gene mutation related to DNA damage repair. BRJ mice are expected to tolerate DNA damage compared with NOG mice, because BRJ mice do not have the *prkdc* gene mutation. As expected, BRJ mice treated with Dox showed significantly long survival compared with NOG mice. Thus, BRJ mice are considered to be useful for experiments of DNA-damaging oncotherapies, for example, radiotherapy or antibody conjugated with DNA damage inducers. Effect of some approved antitumor drugs was also able to be evaluated in PDX-bearing BRJ mice. In conclusion, we established a drug evaluation system using PDX-bearing BRJ mice and it is expected to contribute to development of cancer therapies.