In vivo antitumor evaluation model for immune checkpoint inhibitors

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Mar. 2024

Summary

- CIEM* has established a method that is more sensitive than conventional methods in *in vivo* antitumor evaluation experiments of immune checkpoint inhibitors (hereinafter referred to as ICI**) using HSC***engrafted humanized mice.

- **Background** :
  - Residual innate immunity (especially macrophages) in severely immunodeficient mice may strongly influence the antitumor response of ICI. Therefore, it has been difficult to accurately detect antitumor responses by human immune cells.
  - By using FcResolv NOG (NOG-FcgR knockout) mice in which murine macrophage activity was suppressed, it was revealed that the antitumor effects of ICI could be confirmed clearly against some cancer cell lines. Katano et al. *Scientific reports 2021*

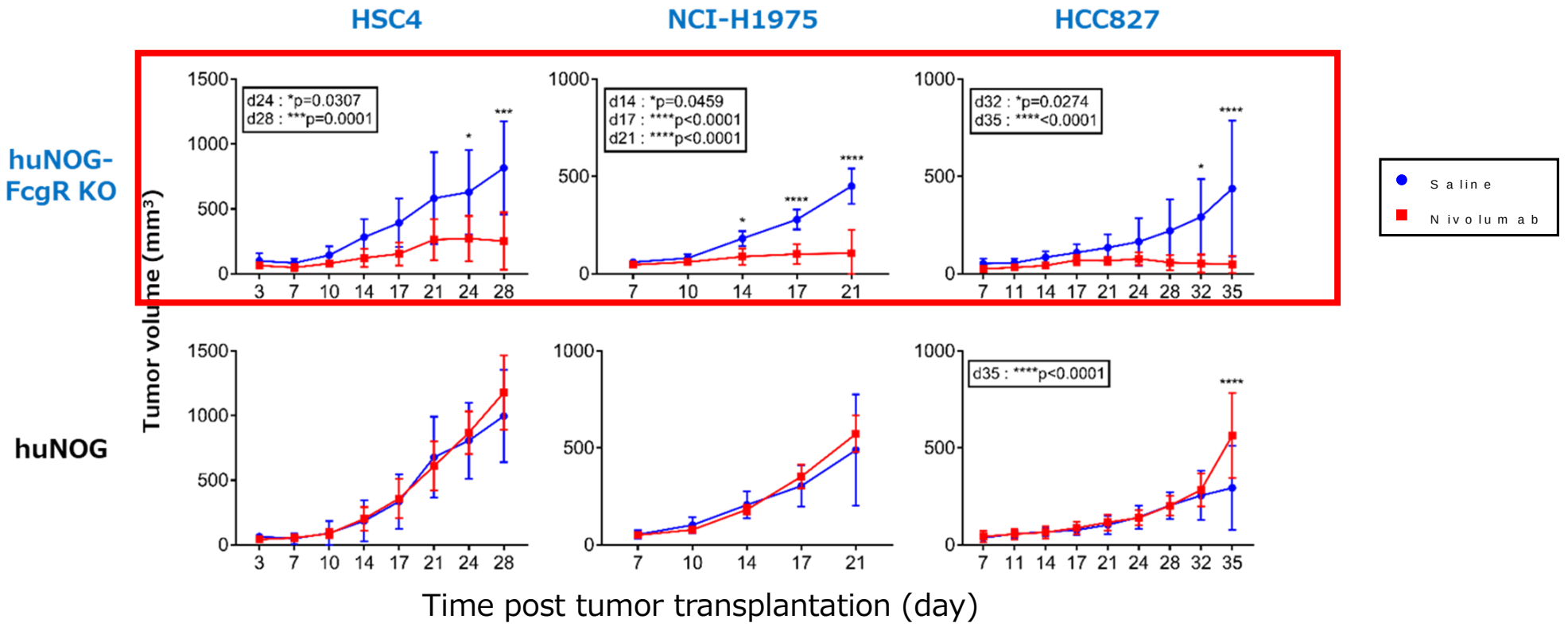
CIEM*: From April 2024, Central Institute for Experimental Animals (CIEA) will be renamed Central Institute for Experimental Medicine and Life Science (CIEM).

ICI** : Immune checkpoint inhibitor, nivolumab is used at CIEM***.

HSC*** : Umbilical cord blood-derived Hematopoietic Stem Cells

Examples of cancer cell lines that can confirm the antitumor effect by ICI (Nivolumab)

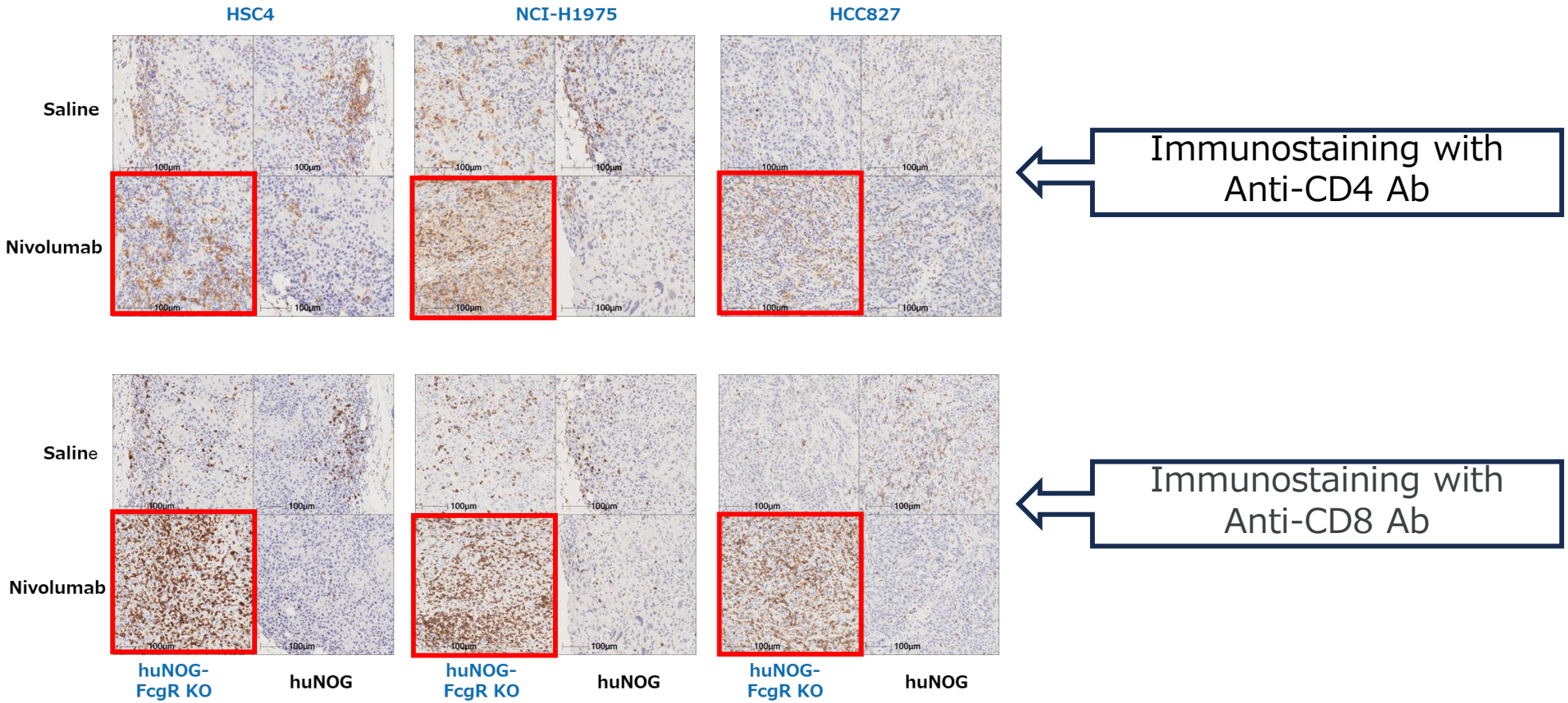
- **Cancer cell lines in which the antitumor effect of nivolumab could be evaluated using FcResolv NOG mice**
- **Head and neck squamous cell carcinoma HSC4, lung adenocarcinoma NCI-H1975, lung adenocarcinoma HCC827**



Katano et al. *Scientific reports 2021*

Examples of cancer cell lines that can confirm the antitumor effect by ICI

—Image : human T cell infiltration in tumor tissue—



In FcResolv NOG mice, an increase in human tumor-infiltrating T cells (TILs) was observed after Nivolumab administration (red frame)

Katano et al. *Scientific reports 2021*

Immune checkpoint inhibitor : *in vivo* evaluation model experimental protocol

Humanized mice production

ICI evaluation protocol

X-ray
~1.5Gy

CB-CD34⁺ cell
3-5x10⁴ i.v.

Cancer Cell Lines*
s.c.

Nivolumab
200μg i.p.

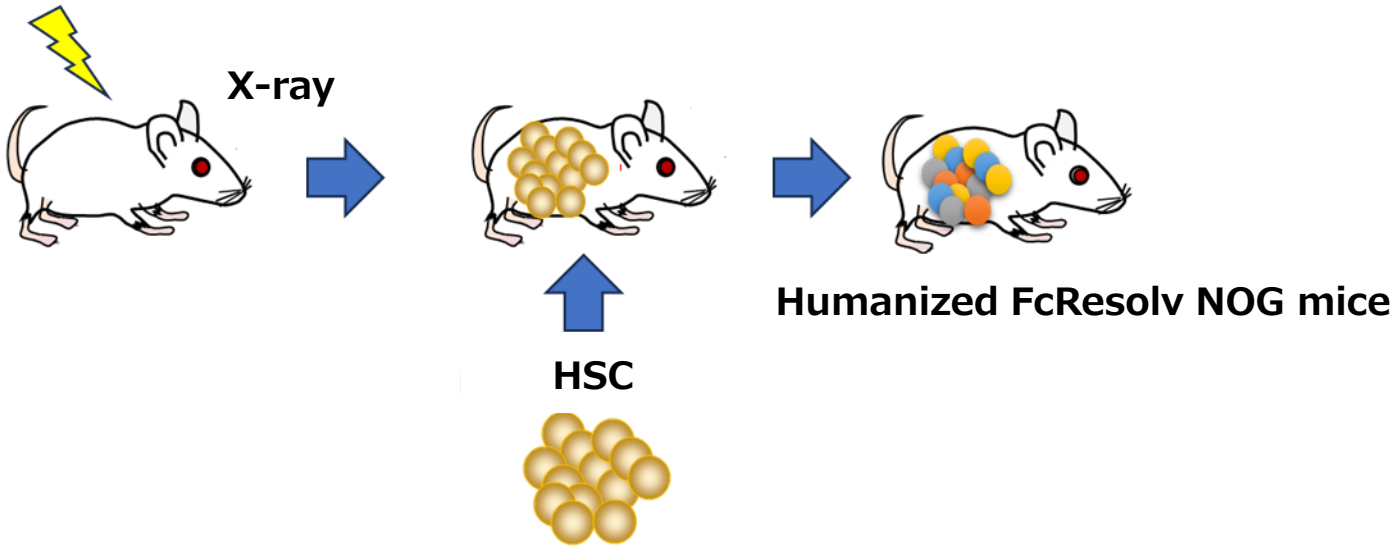
FcResolv NOG
(NOG-FcgR KO)

Endpoint
Autopsy & tumor analysis
✓ FACS analysis
✓ IHC
…etc.

Number of Cancer Cell lines transplanted cells*	
HSC4	1.5×10^6
HCC827	3.0×10^6
NCIH-H1975	3.0×10^6

Katano et al. *Scientific reports 2021*

Method for producing humanized FcResolv NOG mice transplanted with human hematopoietic stem cells (HSC)



- Purchase 6-week-old FcResolv NOG (NOG-FcgR KO) mice and acclimate them for approximately 1 week.
- Mice are irradiated with X-rays to kill some of the mouse-derived bone marrow cells. (If produced at the customer's facility, experiments can also be performed with pretreatment with busulfan.)
- Within 24 hours after X-ray irradiation, 3 to 5 x 10^4 human umbilical cord blood-derived hematopoietic stem cells ($CD34^+$, HSC) are transferred via the tail vein.
- Eleven weeks after HSC transfer, human immune cells are differentiated into peripheral blood.
- At this point, measure the engraftment rate of human leukocytes by flow cytometry.
- Use mice with a human $CD45^+$ cell chimerism rate* of 25% or more.

*Chimerism rate (%) of human $CD45^+$ cells = (number of human $CD45^+$ cells ÷ (human $CD45^+$ cells + mouse $CD45^+$ cells)) × 100