Lack of acute xenogeneic graft-versus-host disease, but retention of T-cell function following engraftment of human peripheral blood mononuclear cells in NSG mice deficient in MHC class I and II expression

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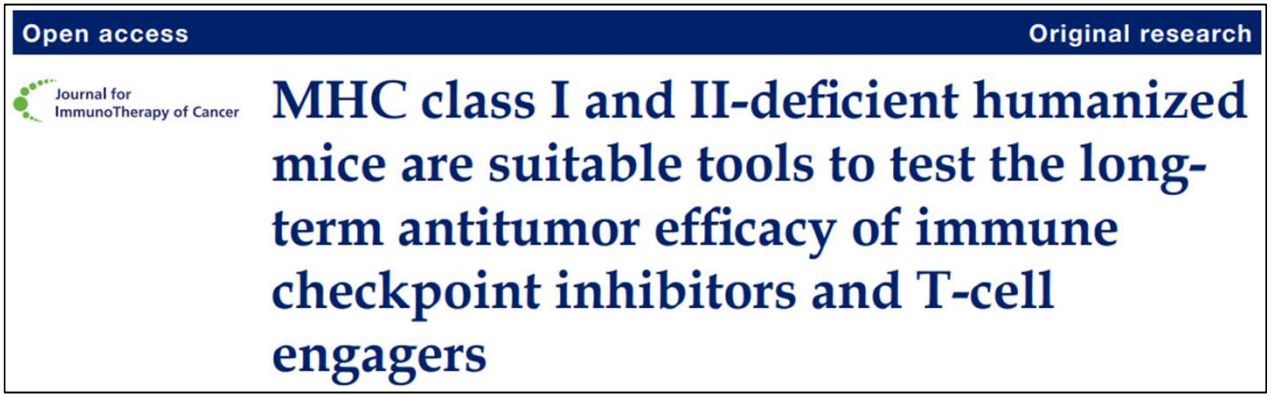
Humanized mouse model



- Journal for ImmunoTherapy of Cancer (IF=10.3 (2023))
- 2024년 9월 accept

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ It has been observed that major histocompatibility complex (MHC) modifications in mice engrafted with human peripheral blood mononuclear cells (PBMCs) significantly reduce xenograft-versus-host disease (xGVHD) severity, the main limitation of PBMC-humanized mouse models. However, the impact of these modifications on the antitumor immune properties and the suitability of these mice for testing the therapeutic potential of immunotherapeutic strategies remain poorly addressed.

WHAT THIS STUDY ADDS

⇒ The absence of severe xGVHD in MHC-dKO mice allows the exploration and characterization of the efficacy of checkpoint inhibitor combinations and T-cell engagers, including long-term immunity.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study demonstrates that humanized MHC-dKO immunodeficient mice allow and refine the preclinical testing of immunotherapy agents for which experimentation is precluded in conventional immunodeficient mice, opening up new possibilities for cancer immunotherapy research.

Humanized mouse model

*APC: antigen-presenting cell
*prkdc : protein kinase, DNA-activated, catalytic polypeptide

- **Humanized mouse model**
- **NSG mouse (or NOG mouse)**→ **immunodeficiency**→ commonly used **model** for studying **human immunity**
  - **NSG**
    - NOD / scid / IL2rg^{null}
  - **NOG**
    - NOD / Shi-scid / IL2rg^{null}
- **NOD** background
  - Non-obese diabetic → **선천 면역↓**
    - impaired NK cell function, complement C5 deficient, APC impairment
- **SCID** (severe combined immunodeficiency)
  - prkdc gene mutation → antigen receptor 다양성에 필수적인 **V(D)J recombination** 의 impairment
    → **functional** 한 **T and B cell** 발달 억제
- **IL2rg^{null}**
  - NK 세포 발달을 완전히 억제, 기존 T, B cell 의 cytokine signaling 저해

Hess, N. J., Brown, M. E., & Capitini, C. M. (2021). GVHD pathogenesis, prevention and treatment: lessons from humanized mouse transplant models. *Frontiers in immunology*, *12*, 723544.
Elhage, A., Sligar, C., Cuthbertson, P., Watson, D., & Sluyter, R. (2022). Insights into mechanisms of graft-versus-host disease through humanised mouse models. Bioscience reports, 42(9), BSR20211986.

Humanized mouse model

*APC: antigen-presenting cell
*prkdc : protein kinase, DNA-activated, catalytic polypeptide

Name	Strain	T	B	NK	Macrophage (for human cells)	Complement
Nude	Foxn1^{null}	No	Yes	Yes	Phagocytose	Yes
Scid	B6.CB17-Prkdc^{scid}/SzJ	No	No	Yes	Phagocytose	Yes
BRG	BALB/c.Rag2$^{-/-}$ IL-2Rg$_c^{-/-}$	No	No	No	Partial tolerant	Yes
NOD-scid	NOD.CB17-Prkdc^{scid}/J	No	No	Function impaired	Tolerant	No C5
NOD/SCID B2m^{null}	NOD.Cg-B2m^{tm1Unc}Prkdc^{scid}/SzJ	No	No	Function loss	Tolerant	No C5
NSG	NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ	No	No	No	Tolerant	No C5
NOG	NOD.Cg-Prkdc^{scid}IL2rg^{tm1Sug}/JicTac	No	No	No	Tolerant	No C5
BRGS	BALB/c.Rag2$^{-/-}$IL-2Rg$_c^{-/-}$NOD.sirpa	No	No	No	Tolerant	Yes
hSIRPa-BRG	BALB/c.Rag2$^{-/-}$IL-2Rg$_c^{-/-}$human.sirpa	No	No	No	Tolerant	Yes

Tian, H., Lyu, Y., Yang, Y. G., & Hu, Z. (2020). Humanized rodent models for cancer research. Frontiers in Oncology, 1696.

Humanized mouse model

*SRC: stem repopulating cell (=HSC)

- **Hu-PBL**
- **Hu-HSC** (SRC)
- **Hu-BLT**

	Hu-PBL-SCID	SRC-Hu	Thy/HSC (BLT)
Accessibility of human sample	Good	Moderate	Difficult (potential ethic problem)
Technique for model construction	Easy	Moderate	Relative difficult (required anesthesia and transplant technique)
Human immune cell survival/development	Majority of activated human T cells; Transient human B cells, myeloid cells and NK cells;	Multi-lineage human immune cell reconstitution; Poor human thymopoiesis; Lack of HLA mediate thymic selection for human T cells.	Multi-lineage human immune cell reconstitution; Robust human thymopoiesis; Human TCR repertoire influenced by mouse antigen.
Human immune function	T cell responses; Lack of interaction between human T cells, B cells, and myeloid cells.	Poor HLA restricted T cell responses; Poor T cell-dependent humoral responses.	Good HLA restricted T cell responses; Good T cell-dependent humoral response.
Time window	Short	Long	Long

Guil-Luna, S., Sedlik, C., & Piaggio, E. (2021). Humanized mouse models to evaluate cancer immunotherapeutics. Annual Review of Cancer Biology, 5, 119-136.

Humanized mouse model (Hu-PBL)

*SRC: stem repopulating cell (=HSC)
*PBL: Peripheral Blood Lymphocyte

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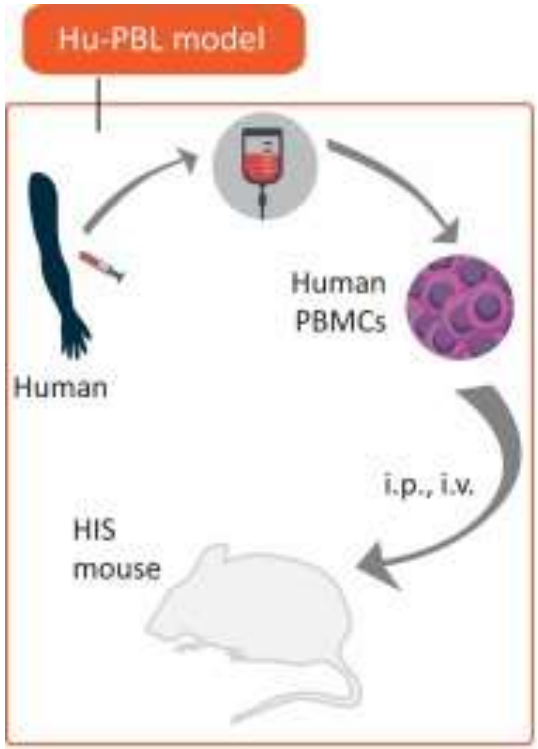

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Humanized mouse model (Hu-HSC)

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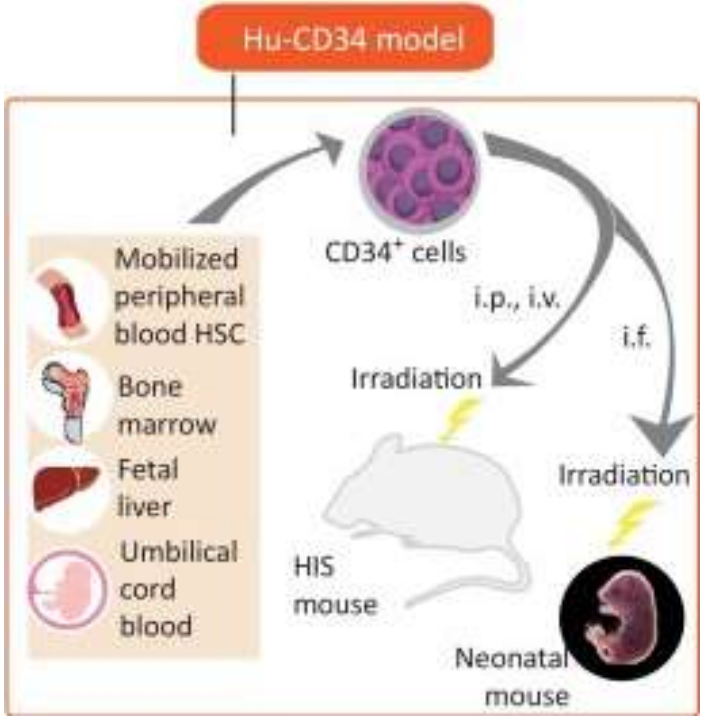
	SRC-Hu
Accessibility of human sample	Moderate
Technique for model construction	Moderate
Human immune cell survival/development	Multi-lineage human immune cell reconstitution; Poor human thymopoiesis; Lack of HLA mediate thymic selection for human T cells.
Human immune function	Poor HLA restricted T cell responses; Poor T cell-dependent humoral responses.
Time window	Long



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Humanized mouse model (Hu-BLT)

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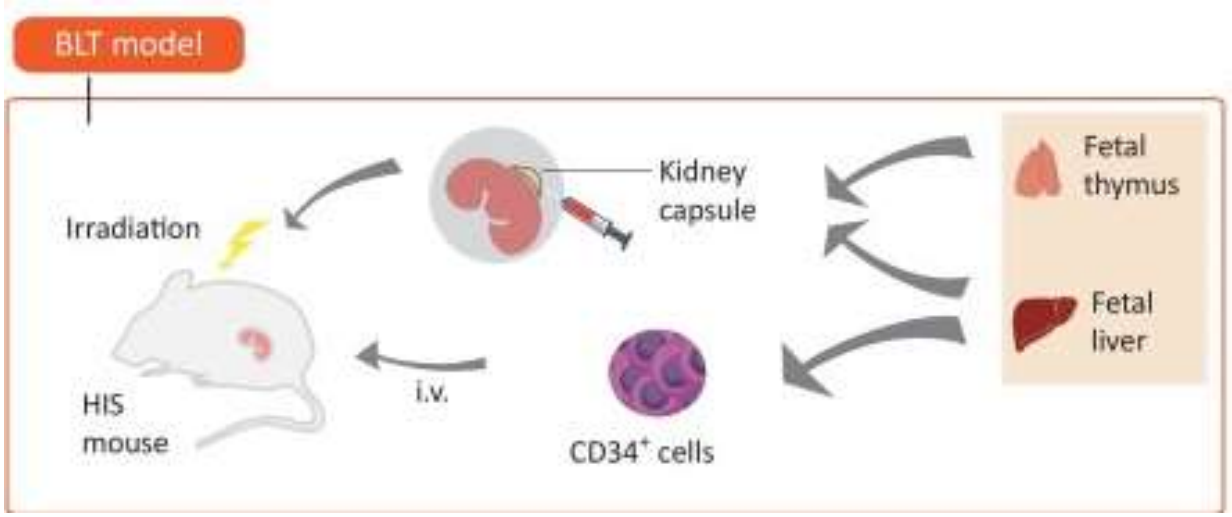
	Thy/HSC (BLT)
Accessibility of human sample	Difficult (potential ethic problem)
Technique for model construction	Relative difficult (required anesthesia and transplant technique)
Human immune cell survival/development	Multi-lineage human immune cell reconstitution; Robust human thymopoiesis; Human TCR repertoire influenced by mouse antigen.
Human immune function	Good HLA restricted T cell responses; Good T cell-dependent humoral response.
Time window	Long



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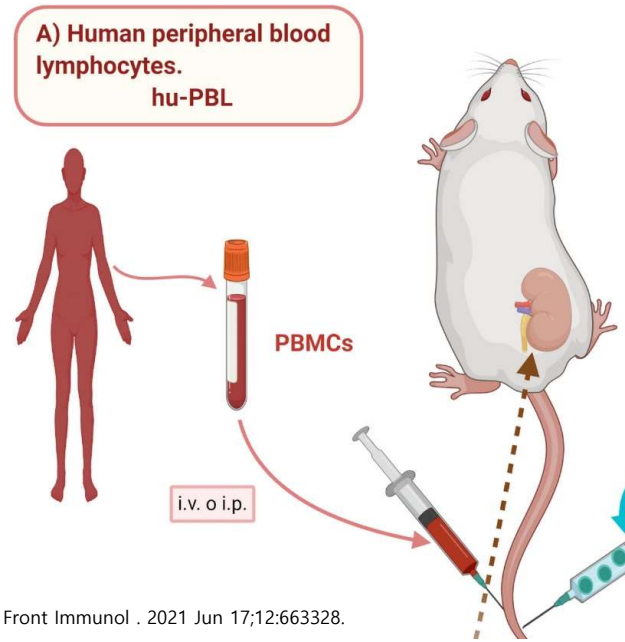
Introduction

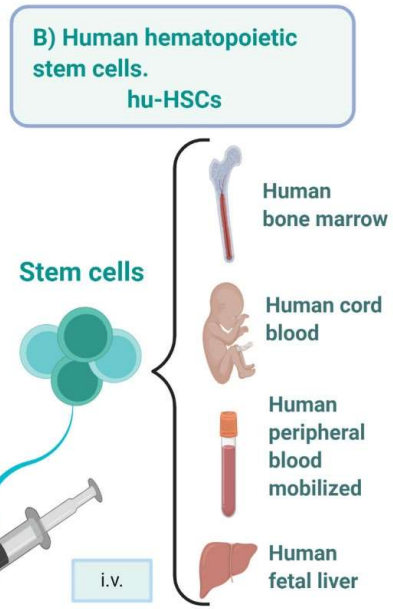
*G-CSF: granulocyte colony-stimulating factor
*SCID: severe combined immunodeficiency

Hu-PBL model



- Easy to establish
- Efficient T cell engraftment
- Recall responses of human cell donor
- Poor myeloid cell engraftment
- Lethal xenogeneic GvHD

Hu-HSC model



- Development of multiple hematopoietic lineages
- Primary immune responses
- Human T cells restricted to murine MHC

Front Immunol . 2021 Jun 17;12:663328.

blog.crownbio.com

Hu-PBL (peripheral blood leukocyte) models

- Generated by injection of mice with mature lymphoid populations including **peripheral blood mononuclear cells (PBMC)**, lymph node cells, and splenocytes.
- For immuno-oncology studies, PBMC are the most commonly used source of PBL

Hu-HSC (hematopoietic stem cell) models

- Generated using human **CD34+ hematopoietic stem cells** (HSC), also called SCID-repopulating cells, derived from bone marrow, umbilical cord blood, fetal liver, or G-CSF-mobilized peripheral blood.

Introduction

*HLA: human leukocyte antigen
*xGvHD: xenogeneic graft-versus-host diesase

- **Hu-HSC**
  - 한계: **이식한 면역세포**(특히 T 세포) 가 성숙하기 위해 필요한 thymus 가 부재
- **Hu-BLT**
  - 한계: 모델 구축의 **어려움**, **불완전한** 성숙
- **Hu-PBL**
  - 성숙한 면역세포로 구성
  - 한계: **NSG 마우스**에 이식 시 **xGvHD 발생** (주로 acute GvHD)

    → **donor immune cell** 이 NSG 마우스의 **MHC I, II**를 인식하여 activation 되기 때문

    → **acute GvHD** 에서 **MHC I** 은 **cytotoxic T cell (CD8$^+$)** 의 활성화를 일으켜 **host cell** 의 systemic 한 **destruction** 유발
    → **acute GvHD** 에서 **MHC II** 는 **helper T cell (CD4$^+$)** 의 활성화를 일으켜 **inflammatory cytokine** 분비 및 **CRS** 유발

  - 따라서 **Hu-PBL** 에서 **GvHD 를 최소화**할 수 있는 다양한 방법이 요구됨

Introduction

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- **Hu-PBL** 에서 **GvHD** 를 **최소화**할 수 있는 **다양한 방법**이 요구됨
  ① graft 에서 xenoreactive 면역반응을 일으키는 특정 cell population (CD4⁺) 을 **제거** 후 이식
  ② 이식 후 cyclophosphamide (면역 억제제 투여)하여 GvHD 억제 시도
  ③ MHC class I and II double knock-out NSG host (**"MHC-dKO NSG"**) 사용
    - 이식 결과: aGvHD 없으면서도 anti-tumor activity 유지
    - 이중 항체 (항암제) 사용 결과: aGvHD 없으면서도 tumor regression 보임

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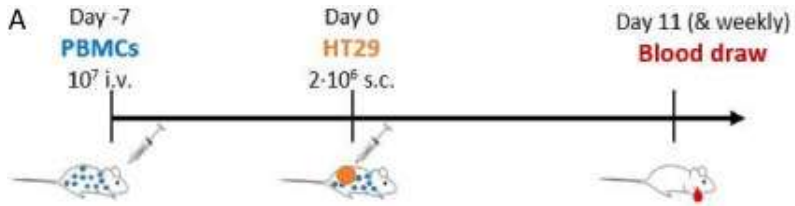
Fig 1. CD4+ T-cell depletion from the PBMC inoculum significantly reduces xGHVD but also abrogates antitumor activity

① graft 에서 xenoreactive 면역반응을 일으키는 특정 cell population ($CD4^+$) 을 **제거** 후 이식

- **선행 연구 결과**
  - T cell 면역억제제 연구에서,
    $CD4^+$ T cell 결핍 PBMC 이식 시 → 다른 세포군 이식에 비해 xGvHD 덜하다는 것 발견
- **Figure 1**
  - $CD4^+$ T cell 결핍 PBMC → HT29 유래 tumor-bearing NSG 마우스에 이식
    (HT29: colorectal cancer line)



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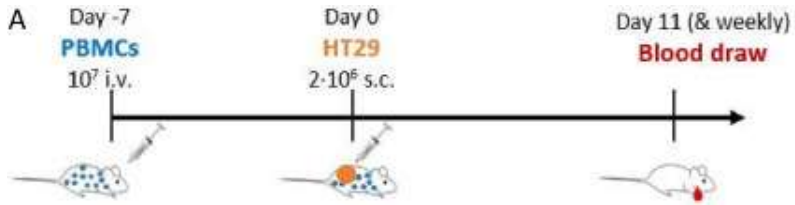
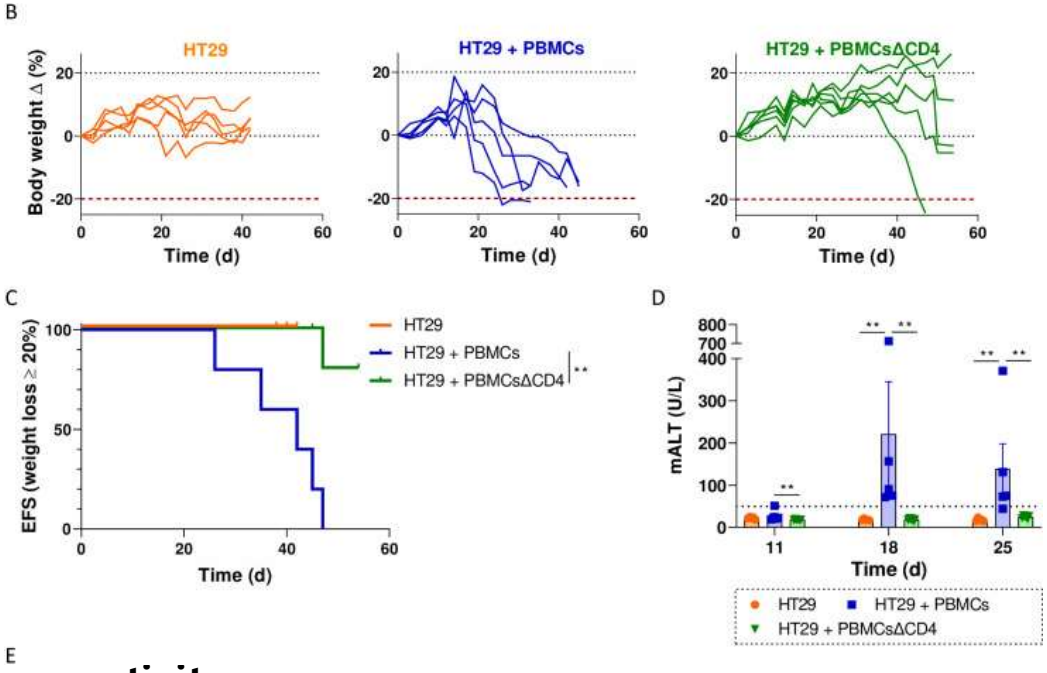


- **Results**
  - **xGvHD development** is dependent to **$CD4^+$ T-cell**
    - $CD4^+$ T-cell depletion → body weight loss ↓
    - $CD4^+$ T-cell depletion → liver toxicity (mALT) ↓
  - human immune cell activation → murine plasma 에서 hIFN-γ 측정 → 마찬가지로 $CD4^+$ cell depletion 에 의해 함께 감소



- **$CD4^+$T cells are critial for xGvHD, but also for antitumor activity**

EFS: event-free survival
mALT: mouse alanine aminotransferase

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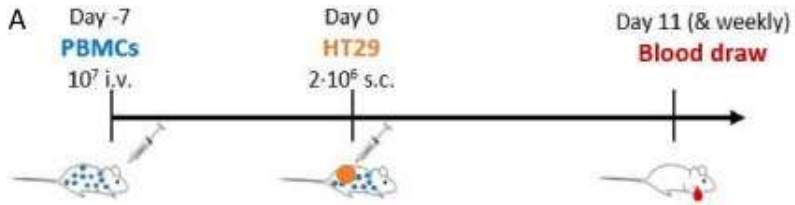

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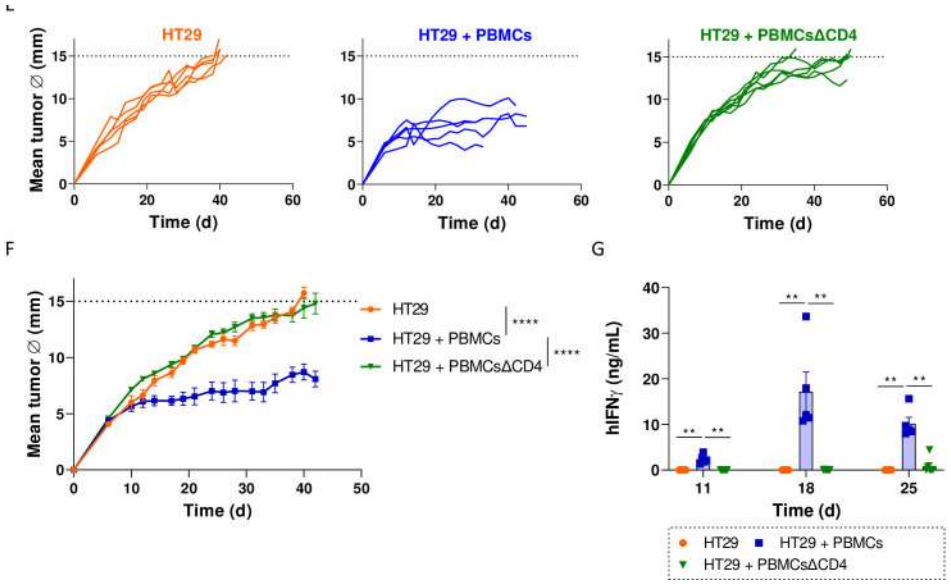
    - $CD4^+$ T-cell depletion → body weight loss ↓
    - $CD4^+$ T-cell depletion → liver toxicity (mALT) ↓
  - **loss** of **anti-tumor effect** is proportional to **$CD4^+$ T-cell depletion**
    - $CD4^+$ T-cell depletion → tumor diameter ↑
    - $CD4^+$ T-cell depletion → human immune cell activation ↓ (murine plasma 에서 hIFN-γ 측정)



→ $CD4^+$T cells are critial for xGvHD, but also for antitumor activity

EFS: event-free survival
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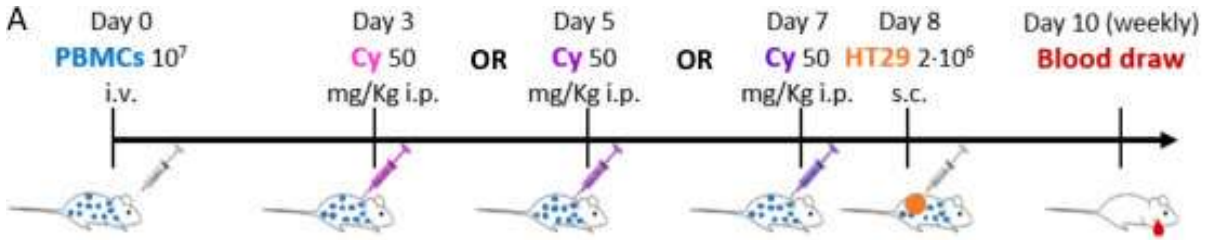
Fig 2. Post-transplantation cyclophosphamide does not significantly reduce xGVHD and curbs antitumor activity

② 이식 후 cyclophosphamide (면역 억제제 투여)하여 GvHD 억제 시도

- **선행 연구 결과**
  - 임상에서 allogeneic BMT(골수이식) 수 일 후 → 고용량의 cyclophosphamide 투여 시 → GvHD 발병률을 유의적으로 감소한다는 연구 결과
    - 특히 가장 증식이 활발하고 alloreactive한 T-cell clones 들이 target 됨이 알려짐
- **Figure 2**
  - post-PBMC engraftment → intraperitoneal(i.p.) **cyclophosphamide (Cy)** administration



→ xenoreactive T-cell 만 depletion 되는지? → anti-tumor effect 는 유지하면서, xGvHD 만 감소하는지?

  - Cyclophosphamide
    - 50 mg/kg (tolerable & active dose)
    - different schedules (day +3, +5, +7 post PBMC engraftment)
    - tumor cell 에는 영향이 없는 것 확인 (day +7 기준)

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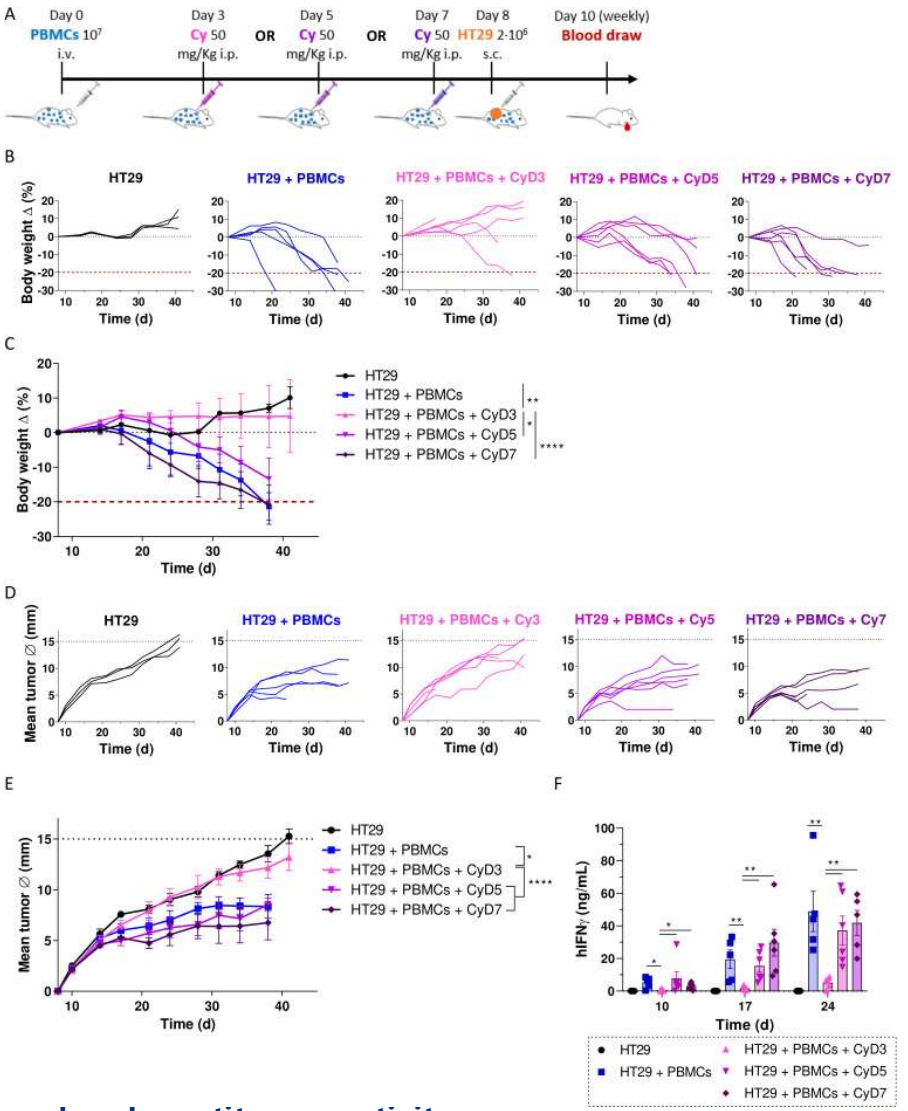
- **Figure 2**
  - post-PBMC engraftment
    → intraperitoneal(i.p.) **cyclophosphamide (Cy)** administration
- **Results**
  - **Early cyclophosphamide (day+3)**
    - xGvHD-associated body weight loss ↓
    - 비정상적 수치의 mALT incidence ↓
    - 그러나...
      - anti-tumor effect ↓ (proportional)
      - plasma hIFN-γ ↓
  - **Intermediate-term cyclophosphamide (day+5)**
    - anti-tumor effect retained
    - xGvHD-associated body weight loss 완화X , plasma hIFN-γ 변화 X
  - **Late-term cyclophosphamide (day+7)** → no significant effect



→ Post-transplantation cyclophosphamide does not significantly reduce xGVHD and curbs antitumor activity

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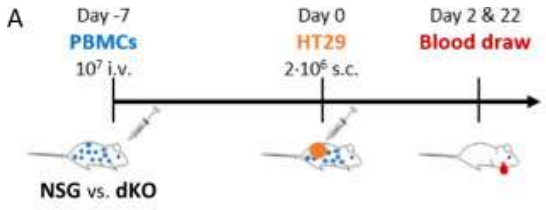
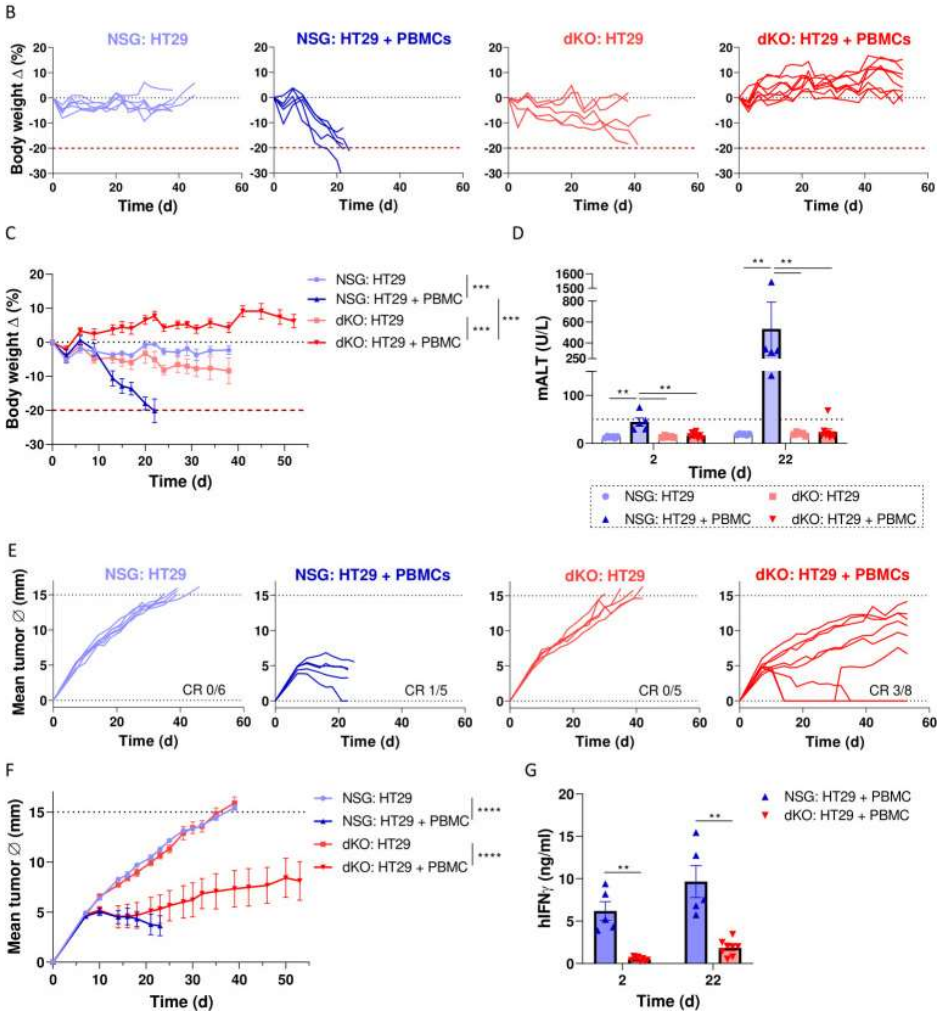
Fig 3. Severe xGVHD is abrogated in MHC-dKO NSG mice ($(K^bD^b)^{null}$ $(IA)^{null}$), while the antitumor effect is preserved

③ MHC class I and II double knock-out NSG host (“**MHC-dKO NSG**”) 사용

- **Figure 3**
  - **NSG**
  - **MHC-dKO NSG**
  - Both groups of mice were co-engrafted with human HT29 carcinoma cells and human PBMCs



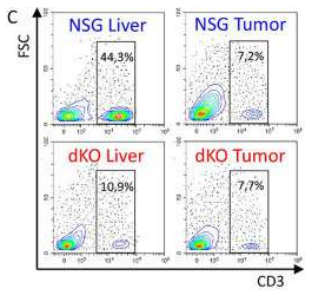
- **Results**
  - **NSG mice** → xGvHD
    - xGvHD-associated body weight loss
    - plasma mALT ↑ (day2 , day 22)
  - **MHC-dKO NSG mice** → no xGvHD
    - No xGvHD-associated body weight loss
    - No changes in plasma mALT
  - Both groups exhibited **anti-tumor activity** (NSG limited lifespan)
  - detectable plasma hIFN-γ in both groups (5-fold in NSG)

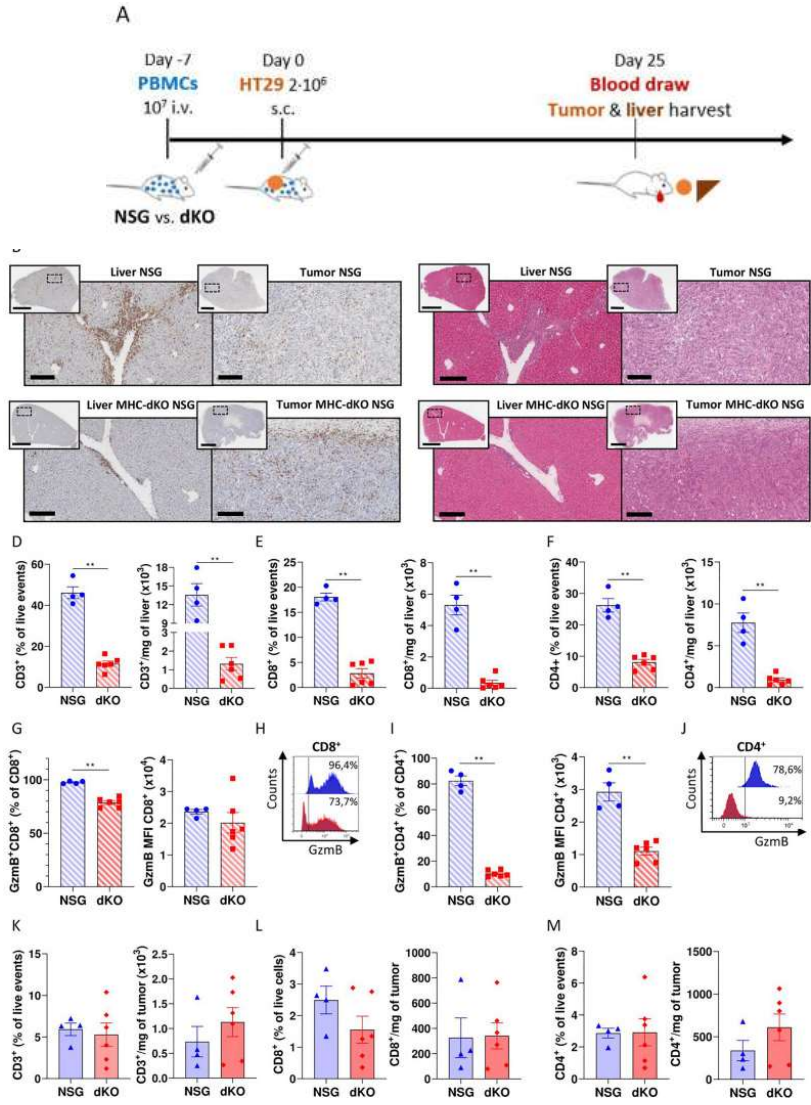


→ **MHC class I and II gene deletions uncouple antitumor efficacy and xGVHD toxicity phenomena**

Fig 4. T-cell density and activation are attenuated in MHC-dKO NSG mice livers, while the tumor immune infiltrate remains comparable

- **Figure 4**
  - Sacrifice mice on day +25 → analyze liver and tumor immune infiltrates
- **Results**
  - Liver human CD3⁺, CD4⁺, CD8⁺, T-cell infiltration
    - **higher in NSG** compared to MHC-dKO-NSG
  - Both CD4⁺ and CD8⁺ cells
    - **more intense expression** of cytotoxic marker **granzyme** B in **NSG**



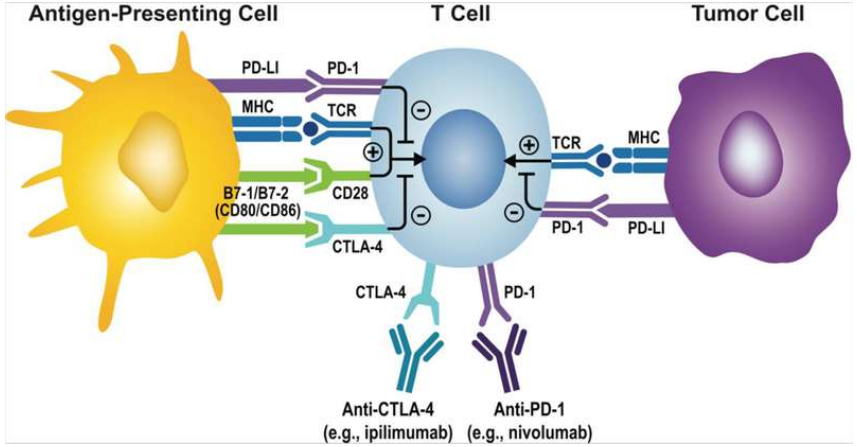


→ MHC silencing in the NSG mouse model is associated with a significant decrease in liver inflammation, while the abundance and overall phenotypic characteristics of TILs are preserved

TIL: tumor-infiltrating lymphocyte

Fig 5. The humanized MHC-dKO NSG model enables the antitumor activity characterization of clinical-grade immune checkpoint inhibitors nivolumab plus ipilimumab

- **Figure 5**
  - **Immume checkpoint inhibitor**
    - **Ipilimumab** (anti PD-1)
    - **Nivolumab** (anti-CTLA-4)



- **Evaluation** of **antitumor effect** of **clinical-grade immunotherapy** testing the combination of **nivolumab** and **ipilimumab** in **NSG** and **MHC-dKO NSG** mice

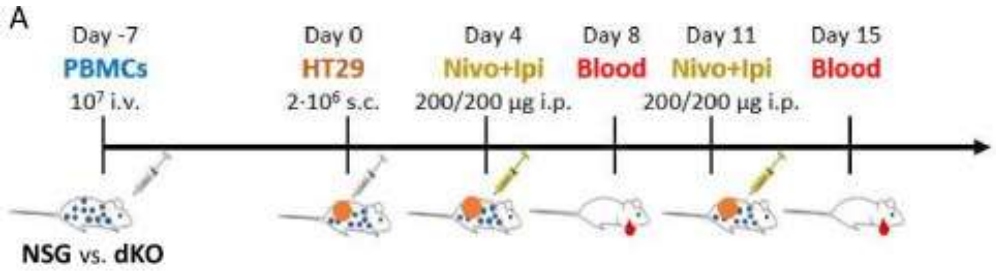
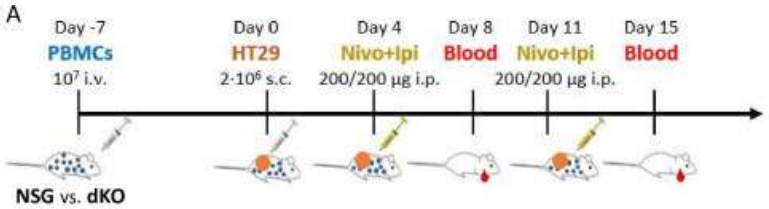


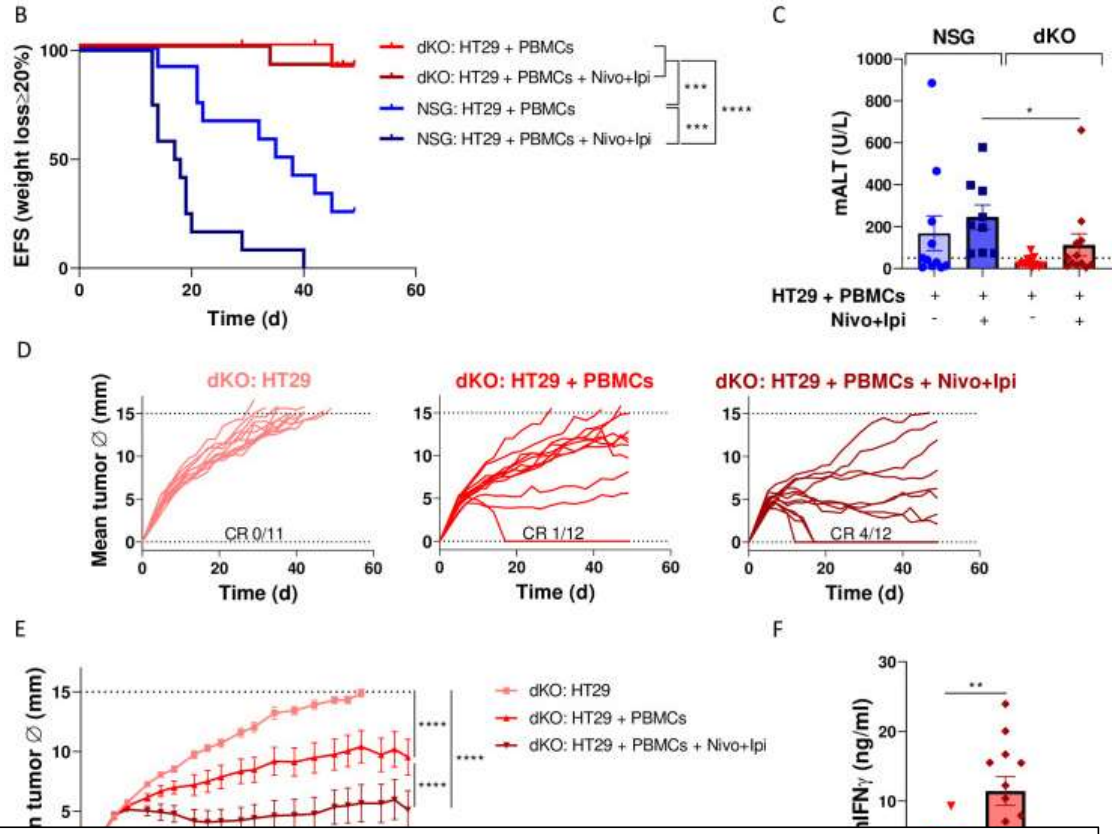
Fig 5. The humanized MHC-dKO NSG model enables the antitumor activity characterization of clinical-grade immune checkpoint inhibitors nivolumab plus ipilimumab

EFS: event-free survival
mALT: mouse alanine aminotransferase
CR: complete response rate
Ipi: Ipilimumab
Nivo : Nivolumab

- **Figure 5**
  - **Evaluation** of the efficacy of **immune checpoint inhibitors** in **NSG** and **MHC-dKO NSG** mice



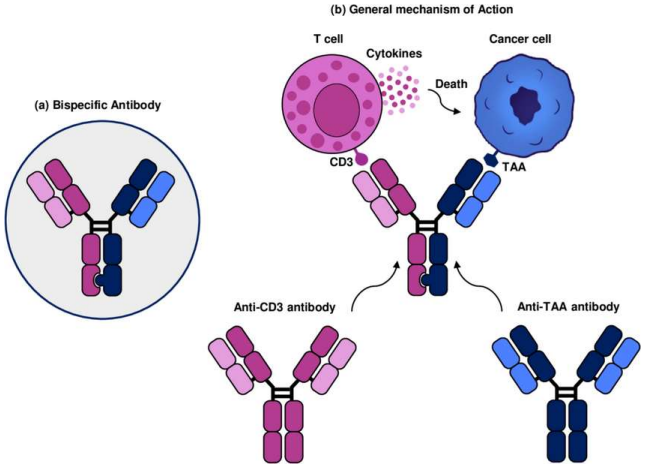
- **Results**
  - **NSG mice** → xGvHD
    - xGvHD-associated body weight loss (limited follow-up)
    - plasma mALT ↑
  - **MHC-dKO NSG mice** → no xGvHD
    - No xGvHD-associated body weight loss
    - No elevation in plasma mALT (day 15 : nonsignificant)
  - In both groups PBMCs exhibited **anti-tumor activity**
  - **immune checpoint inhibitors** enhanced **anti-tumor activity**



→ MHC-dKO NSG mice enabled the observation of profound and durable antitumor immune responses to nivolumab plus ipilimumab that were not assessable in NSG mice due to accelerated xGVHD

Fig 6. The humanized MHC-dKO NSG model enables long-term examination of antitumor effects of a T-cell engager

- **Figure 6**
  - Bispecific anti-CD3 agents
    - **αEpCAM/CD3 BsAb**
  - 항암 효과를 가진 이중항체 (linker effect)



- **Evaluation** of the efficacy of an **αEpCAM/CD3 BsAb** in **NSG** and **MHC-dKO NSG** mice

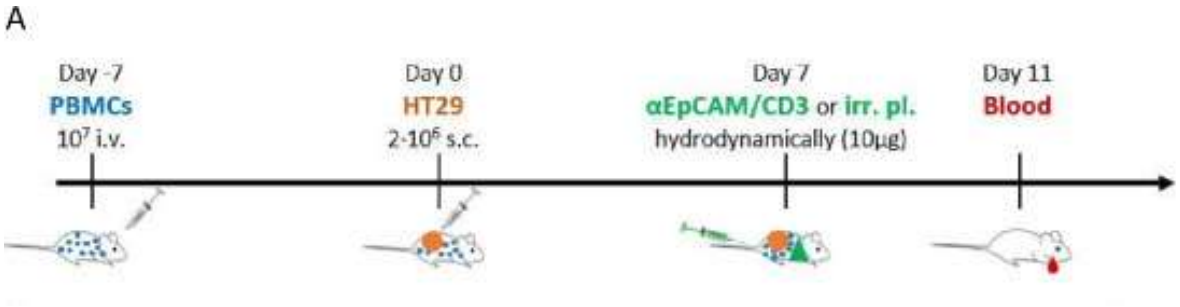
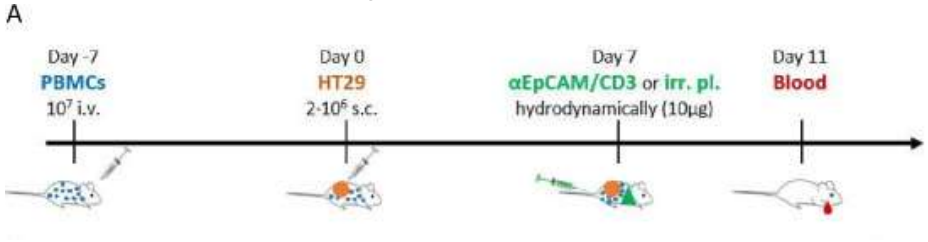


Fig 6. The humanized MHC-dKO NSG model enables long-term examination of antitumor effects of a T-cell engager

EFS: event-free survival
mALT: mouse alanine aminotransferase
CR: complete response rate
Irr. pl. : irrelevant plasmid (B12/CD3 bispecific antibody

- **Figure 6**
  - **Evaluation** of the efficacy of an **αEpCAM/CD3 BsAb** in **NSG** and **MHC-dKO NSG** mice



- **Results**
  - **NSG mice** → xGvHD
    - significantly **shorter** ≥20% weight loss-free survival (EFS)
    - plasma mALT **increase** 4 days after αEpCAM/CD3 BsAb treatment (**higher** elevation in NSG)
  - **MHC-dKO NSG mice** → no xGvHD
    - No xGvHD-associated body weight loss
    - No elevation in plasma mALT attributable to treatment
    - In both groups PBMCs exhibited **anti-tumor activity** (NSG limited lifespan)

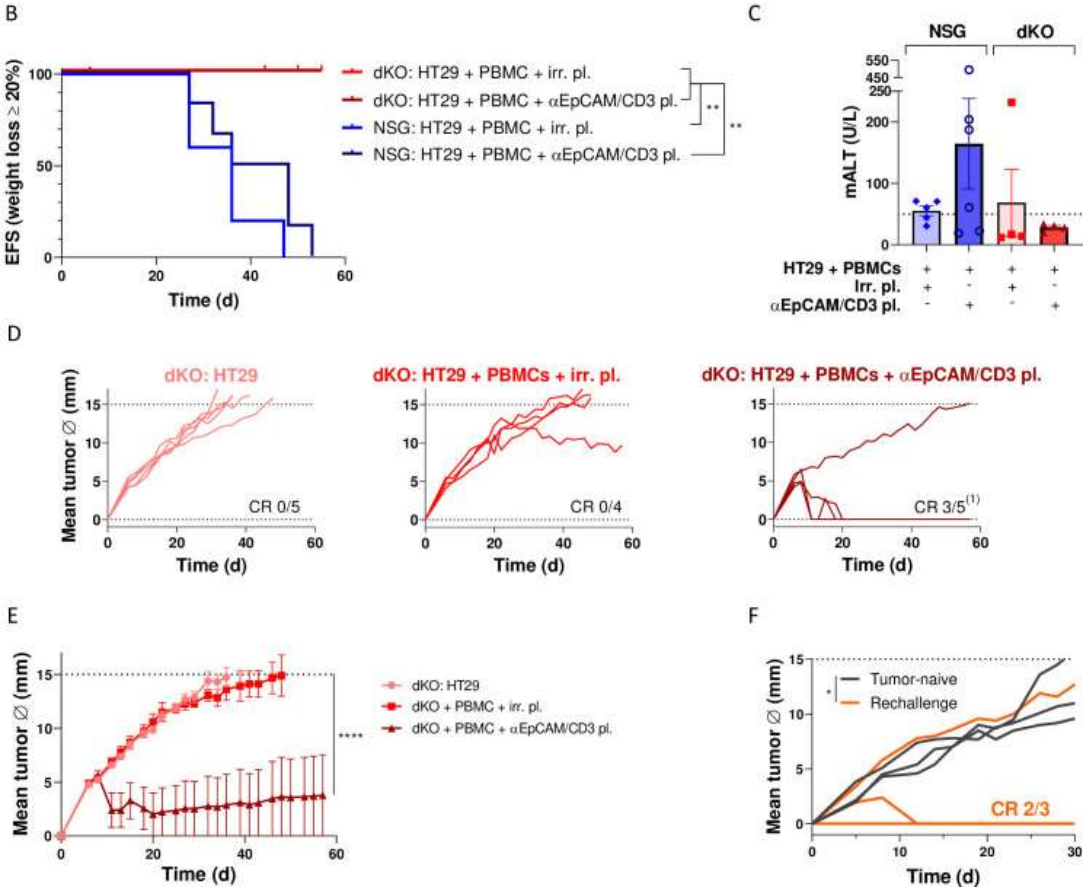


Fig 6. The humanized MHC-dKO NSG model enables long-term examination of antitumor effects of a T-cell engager

EFS: event-free survival
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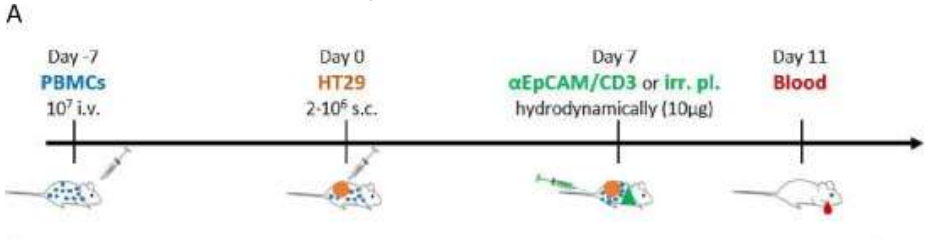
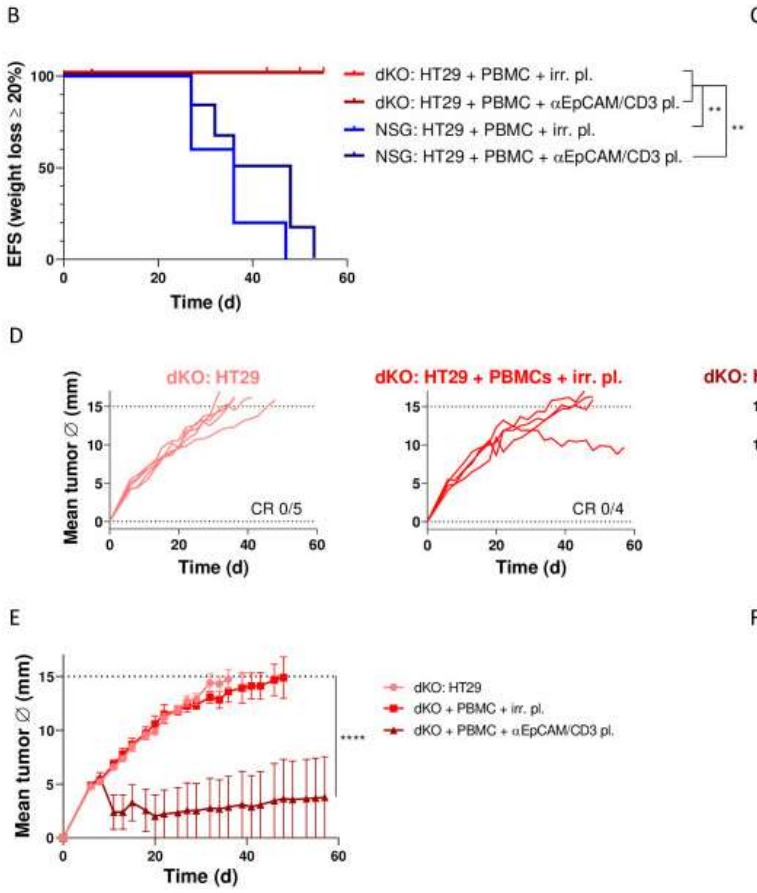
- **Figure 6**
  - **Evaluation** of the efficacy of an **αEpCAM/CD3 BsAb** in **NSG** and **MHC-dKO NSG** mice



- **Results**
  - **Rechallenge** to **HT29 tumor**
    - 30 days after complete response(CR) (day +54)
    - 3 mice, tumor-naïve mouse as control
  - HT29 tumor growth was observed in control mice, while two out of three rechallenged mice spontaneously rejected the new tumor inoculi

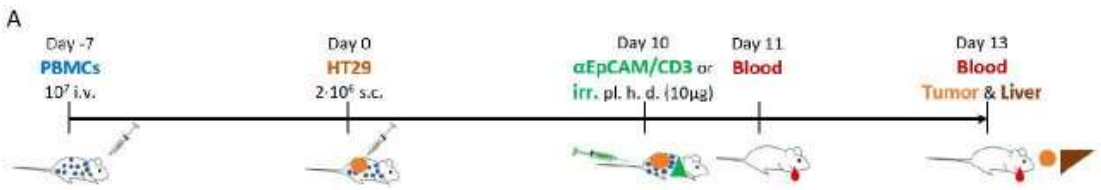


→ **MHC-dKO NSG mice can be used to characterize T-cell engager strategies and suggest that αCD3BsAb might be capable of inducing protective long-term immune responses**

Fig 7. The PBMC-humanized MHC-dKO NSG model enables the pharmacodynamic characterization of a T-cell engager

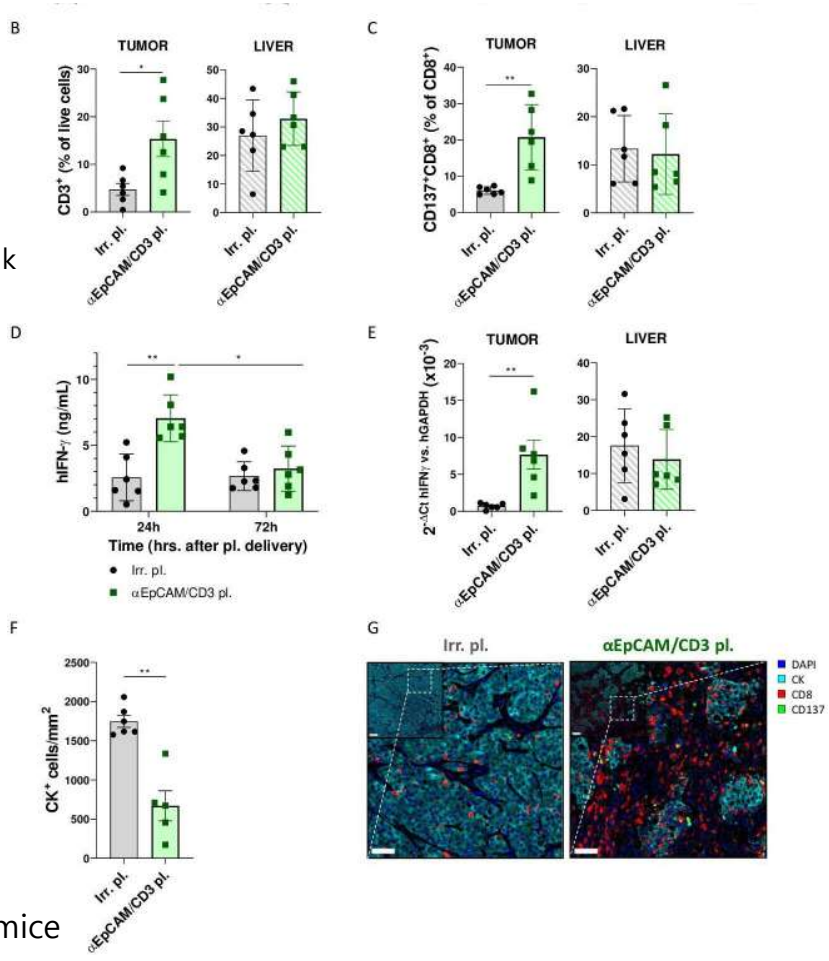
TIL: tumor infiltrating lymphocyte

- **Figure 7**
  - **Characterization** of the **pharmacodynamics** of **αEpCAM/CD3 BsAb** in **NSG** and **MHC-dKO NSG** mice



  - In this case, mice treated with BsAb at day +10 and sacrificed at the time tumors started to shrink (day +20 post-PBMC engraftment and +72-hour post-αEpCAM/CD3 BsAb treatment)

- **Results**
  - mice treated with the **αEpCAM/CD3 BsAb**
    → significantly higher abundance of **CD3+ TILs** as compared with control mice
    - predominantly dependent on CD8+ T cells
  - **Higher CD137** membrane expression within the tumor-infiltrating CD8+ T-cell compartment was observed in **treated tumors** but **not** in the **liver**
    - CD137 : a membrane receptor upregulated by CD3+ T-cell activation signaling
  - A **transient** and **significant** elevation of plasma hIFN-γ was observed 24 hours after plasmid administration **only** in αEpCAM/CD3 treated mice



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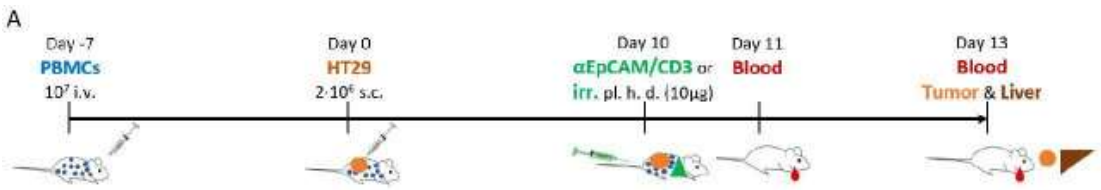

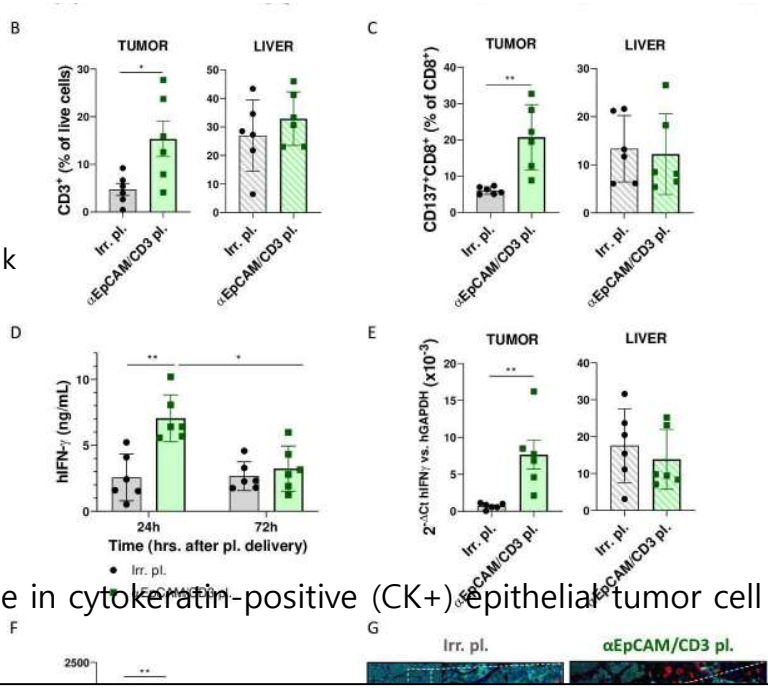

  - In this case, mice treated with BsAb at day +10 and sacrificed at the time tumors started to shrink (day +20 post-PBMC engraftment and +72-hour post-αEpCAM/CD3 BsAb treatment)

- **Results**
  - significant increase in the tumor tissue, but not in the liver, of treated mice was observed
  - multiple immunofluorescence analyses of tumor tissue showed a significant decrease in cytokeratin-positive (CK+) epithelial tumor cell density

→ Overall, we have observed an increase in the number and activation of tumor-infiltrating T cells, which confirms the mode of action of these agents; and reveals CD8+ T-cell upregulation of the co-stimulatory receptor CD137, which as a result may offer a potential target for new-generation tri-specific T-cell engagers.

THANK YOU