Development of an improved humanized patient-derived xenograft, Hu-PDX, mouse model for evaluation of antitumor immune response in lung cancer

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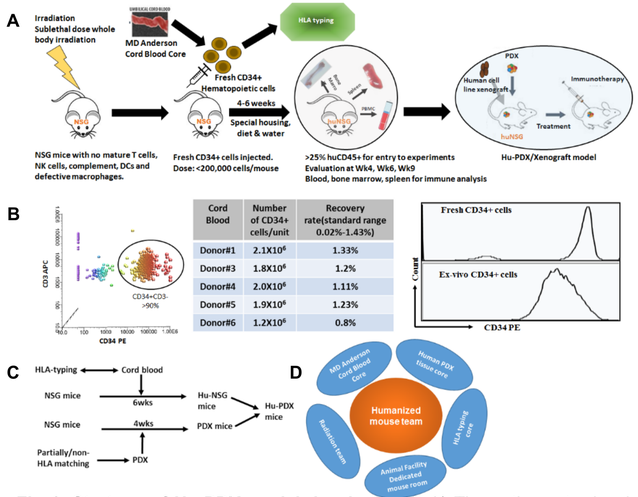
ABSTRACT

Human tumor xenograft models are unable to replicate the human immune system and tumor microenvironment. We developed an improved humanized mouse model, derived from fresh cord blood CD34⁺stem cells (CD34⁺HSCs), and combined it with lung cancer cell line derived human xenografts or patient derived xenografts (Hu-PDXs). Reconstitution of human leukocytes (hCD45⁺) was detected as early as four weeks without the onset of graft vs host disease (GVHD). Repopulated human T, B, Natural Killer (NK), dendritic cells (DC), increased significantly in peripheral blood, spleen and bone marrow. Luciferase-labeled cultured CD34⁺HSC cells were detected in mice, but we were unable to detect reconstituted human immune cells at four weeks, as was observed for fresh CD34⁺HSC. Reconstituted T cells secreted IFN-γ following treatment with phorbol myristate acetate (PMA) or exposure to A549 lung tumor cells, and mediated antigen specific cytotoxic T lymphocyte (CTL) responses, indicating functional activity. Growth of engrafted PDXs and tumor xenografts were not dependent on the human leukocyte antigen (HLA) status of the donor. Treatment with the anti-PD1 checkpoint inhibitor pembrolizumab inhibited tumor growth significantly, and correlated with an increase of CTL and decrease of MDSC levels, regardless of the donor HLA-type. Pembrolizumab had no effect on tumor growth in non-humanized mice. A strong antitumor response to the anti-PD1 inhibitor nivolumab occurred. In conclusion, fresh CD34⁺HSCs are more effective than their expanded counterparts in humanizing mice, and do so in a shorter time. The Hu-PDX model provides an improved platform for evaluation of immunotherapy.

Strategy for development of a humanized PDX mouse model



Cord Blood	Number of CD34+ cells/unit	Recovery rate(standard range 0.02%-1.43%)
Donor#1	$2.1X10^6$	1.33%
Donor#3	$1.8X10^6$	1.2%
Donor#4	$2.0X10^6$	1.11%
Donor#5	$1.9X10^6$	1.23%
Donor#6	$1.2X10^6$	0.8%

Fig 1. Strategy of Hu-PDX model development. A) The pathway to develop Hu-PDX mouse model from fresh cord blood derived CD34⁺ HSCs followed by generation of Hu-PDX mice. B) Purity and recovery of CD34⁺ HSCs from donor cord blood, C) Synchrony of generating Hu-PDX mice, D) integration of institutional multidisciplinary groups for Hu-PDX model development.

Immune profiling of human immune cells in humanized mice

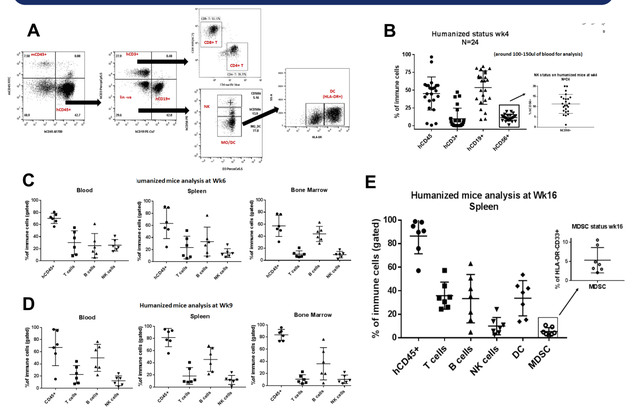


Fig 2. Characterization of humanized mice. A) Gating strategy of multicolor flow cytometry for analysis of human immune cells in mouse organs. B) Analysis of humanization status in PBMC at 4 weeks of post CD34 engraftment. C-D) Analysis of humanized mice at week 6 of post implantation. At this stage, analysis was performed in blood, spleen and bone marrow. E) Myeloid status in humanize mice at week 16.

T cell functionalities and Ag specific T cell response in Hu-PDX

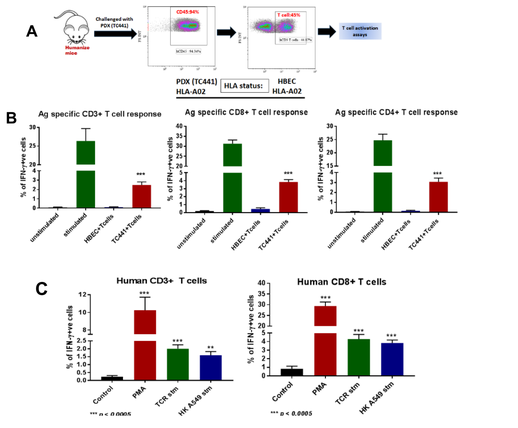


Fig 3. Human T cell functionalities in Hu-PDX. A) Humanized mice were challenged with PDX and four weeks post implantation both spleen and PDX were harvested for in-vitro co-culture assay for intracellular IFN-γ detection. C) PDX Tumor specific T cell response. D) Humanized mice were challenged with A549 NSCLC and T cells functionalities were assessed by intracellular IFN-γ assay.

Human PDX and cell line xenograft tumors in humanized mice

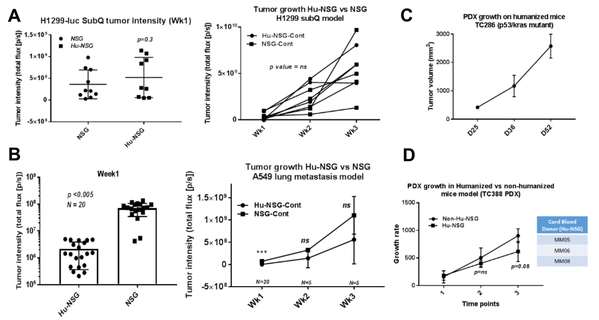


Fig 4. Comparison of tumor growth in humanized vs non-humanized mice. Subcutaneous tumors, experimental lung metastasis and PDXs were grown in humanized mice from multiple donors of different HLA status and tumor growth was compared side by side between humanized and non-humanized mice A) SubQ tumor (H1299), B) Lung metastasis (A549), C-D) human PDX TC286 & TC388 respectively.

Infiltration of human immune cells into humanized and non-humanized PDXs

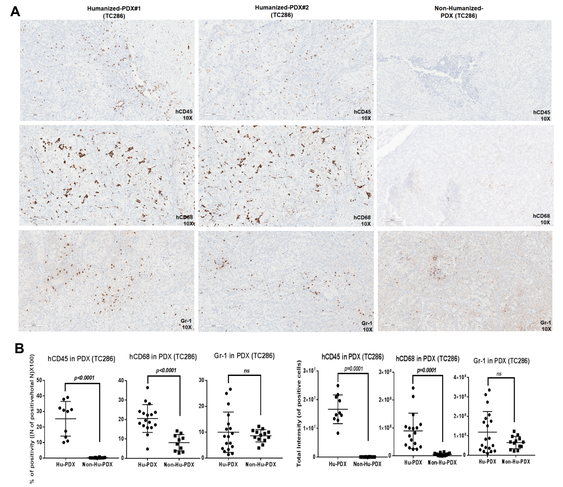


Fig 5. Status of infiltrating human immune cells into Hu-PDXs. A) Immunohistochemistry of human leukocytes (CD45+), macrophages (CD68+), and mouse MDSC (Gr-1+) in PDX (TC286) tumor developed in humanized and non-humanized mice. B) quantitative analysis of human CD45, CD68 and Gr-1 signal in Hu-PDX and Non-Hu-PDX tissue were performed by using ImageScope software.

Antitumor immune effect of pembrolizumab on Hu-PDX and Hu-lung metastasis model.

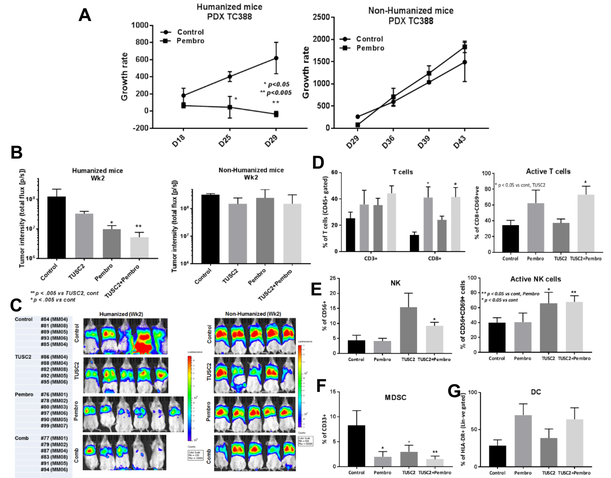


Fig 6. Antitumor effect of Pembrolizumab on Hu-PDX/xenografts. A) Effect of pembrolizumab on Hu-PDX (TC388) developed in Hu-NSG and non-Hu-NSG mice by simultaneous implantation of tumor. B-C) quantitative analysis(B) and IVIS images(C) Antitumor effect of pembrolizumab in combination with TUSC2, an immunogene, on A549-luc metastases. D-G) Immune response analysis elicited by pembrolizumab and in combination with TUSC2 in humanized mice.

CONCLUSIONS

- Humanization with fresh CD34+ HSCs are more effective than their expanded counterparts.
- Reconstitution of human leukocytes (hCD45⁺) was faster and detected as early as four weeks without the onset of GVHD.
- Reconstituted T cells in humanized mice were functionally active and displayed antigen specific CTL response.
- Growth of engrafted PDXs and tumor xenografts were not dependent on the HLA status of the donor.
- Strong antitumor immune responses of anti-PD1 (Pembrolizumab) were found against PDX and xenograft tumors developed in humanized mice but not non-humanized mice.
- The Hu-PDX model provides an improved platform for evaluation of immunotherapy.

References & Disclosures

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