Priority Report

Cancer Research

HER2 CAR-T Cells Eradicate Uveal Melanoma and T-cell Therapy–Resistant Human Melanoma in IL2 Transgenic NOD/SCID IL2 Receptor Knockout Mice

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Abstract

Chimeric antigen receptors (CAR) can transmit signals akin to those from activated T-cell receptors when bound to a cell surface target. CAR-expressing T cells against CD19 can cause curative effects in leukemia and lymphoma and is approved for clinical use. However, no CAR-T therapy is currently approved for use in solid tumors. We hypothesize that the resistance of solid tumors to CAR-T can be overcome by similar means as those used to reactivate tumor-infiltrating T lymphocytes (TIL), for example, by cytokines or immune checkpoint blockade. Here we demonstrate that CAR-T cells directed against HER2 can kill uveal and cutaneous melanoma cells *in vitro* and *in vivo*. Curative effects *in vivo* were only observed in xenografts grown in a NOD/SCID IL2 receptor gamma (NOG) knockout mouse strain transgenic for human IL2. The effect was target-specific, as CRISPR/Cas9-mediated disruption of HER2 in the melanoma cells abrogated the killing effect of the CAR-T cells. The CAR–T cells were also able to kill melanoma cells from patients resistant to adoptive T-cell transfer (ACT) of autologous TILs. Thus, CAR-T therapy represents an option for patients that do not respond to immunotherapy with ACT of TIL or immune checkpoint blockade. In addition, our data highlight the use of IL2 transgenic NOG mice as models to prove efficacy of CAR-T-cell products, possibly even in a personalized manner.

Significance: These findings demonstrate that a novel humanized mouse model can help clinical translation of CAR-T cells against uveal and cutaneous melanoma that do not respond to TIL therapy or immune checkpoint blockade.

Introduction

Immunotherapies have revolutionized the treatment of metastatic malignant cutaneous melanoma by enabling durable responses in a large fraction of patients (1–3). The best effects have been achieved by combination therapies of anti-PD-1 and anti-CTLA4 mABs (4). These target immune checkpoint proteins prevent them from causing T cells to be suppressed, or get exhausted after activation (5). Another immunotherapy showing promising results in smaller trials is adoptive T-cell transfer (ACT) of *ex vivo* expanded tumor-infiltrating T lymphocytes (TIL; ref. 6). Even this therapy can induce durable responses, but the method is laborious, associated with severe toxicity, and thus only performed at few selected sites worldwide. Both ACT and checkpoint inhibitors appear to require preexisting immune reaction mediated via tumor recognition of so-called neoantigens. However, some melanoma, including uveal (ocular) melanoma, express few or no suitable neoantigens and a challenge in the field is how to render these tumors immunogenic. One approach is to use T cells that are engineered to express the CD3 signaling domain fused to either CD28 or 4BB-1 signaling domains and an external receptor targeting a cell surface protein on tumor cells, so called chimeric antigen receptor T cells (CAR-T cell; ref. 7). The first CAR-T cell to be FDA-approved is directed against the CD19 protein expressed on normal and malignant B cells (8). The effects of CAR-T cells are robust and induced durable remissions in a large fraction of children with acute B lymphoblastic leukemia that had failed all other conventional therapies (8). Although this approach is highly encouraging, similar results in solid tumors have not yet been demonstrated.

An obvious hypothesis, based on the knowledge of how ordinary T cells are inactivated by tumors, is that CAR-T cells do not work as well in solid tumors because they become exhausted before they can eradicate the tumor, that the tumor microenvironment block their effect, or that the antigen is not uniformly expressed. Here, we study CAR-T activity in melanoma and find

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means to get durable antitumoral responses in humanized mouse models.

Materials and Methods

Patients and cells

CAR-T cells were generated from peripheral blood mononuclear cells from five different donors by ProMab technologies. The CAR consists of a single-chain variable fragment directed against HER2 fused with a CD8 leader signal, a CD28 transmembrane and activation domain, and a CD3zeta activation domain. TILs and autologous tumor cells came from patients at Herlev Hospital (Herlev, Denmark) either participating or planned to participate in ACT trials and have previously been used in animal experiments (9). 92-1 and MEL202 cells were a kind gift from The European Searchable Tumour Line Database and Cell Bank. Cell authentication of 92-1 cells was performed in 2018 by exome sequencing, confirming a mutation in *GNAQ*. MEL202 have not undergone cell authentication since its purchase in 2015. All cells were screened for *Mycoplasma* using PCR.

In vitro assays

To assess T-cell killing, cell lines expressing luciferase were plated at 2×10^4 cells/well in black 96-well plates (Corning) and cultured in the presence or absence of 6×10^4 CAR-T cells/well. After 48 hours the medium was aspired for IFNγ secretion analysis using an ELISA Kit (Diaclone), and viability of the cancer cells was assessed by measuring luminescence with a GloMax Discover Plate Reader (Promega) after adding luciferin (300 μg/mL) to the cells.

For CRISPR/Cas9 inactivation of HER2, Cas9:crRNA:tracrRNA ribonucleoprotein complex was assembled according to the manufacturer's recommendations (IDT DNA) and transfected to the cells using Lipofectamine RNAiMAX Reagent (Invitrogen). Negative cells were sorted on the basis of absence of HER2-PE antibody staining (clone 24D2; BD Biosciences) using a FACSAria III (BD Biosciences). Absence of HER2 expression in the HER2 knockout cells was confirmed by staining with the same antibody (HER2-PE clone 24D2, BD Biosciences) and analyzed using an Accuri C6 flow cytometer equipped with the BD Accuri C6 software.

Mouse models

All animal experiments were performed in accordance with EU directive 2010/63 and was approved by the regional animal ethics committee (IACUC) of Gothenburg (approval nos. 2014-36, 2016-100, and 2018-1183). Melanoma cells expressing luciferase were transplanted subcutaneously at 2×10^5 cells/mouse with 50% Matrigel (Corning) to NOD/SCID IL2 chain receptor γ knockout mice (NOG mice) or hIL2-NOG mice (Taconic). Patient-derived xenografts (PDX) were produced by subcutaneous serial transplantation to NOG mice. UM121213B is a novel PDX model of uveal melanoma. The melanoma identity of the model was verified by staining the patient biopsy and a PDX tumor biopsy with clinical-grade melanoma antibodies anti-PMEL (HMB45, M0634; Dako), melan A (clone A103; Dako), and anti-S100 (IR504; Dako). HER2 expression or T-cell determination was determined by IHC using a clinical-grade anti-HER2 antibody (A0485) or an anti-CD3 antibody (A0452), both from Dako. T cells were also phenotyped by flow cytometry using

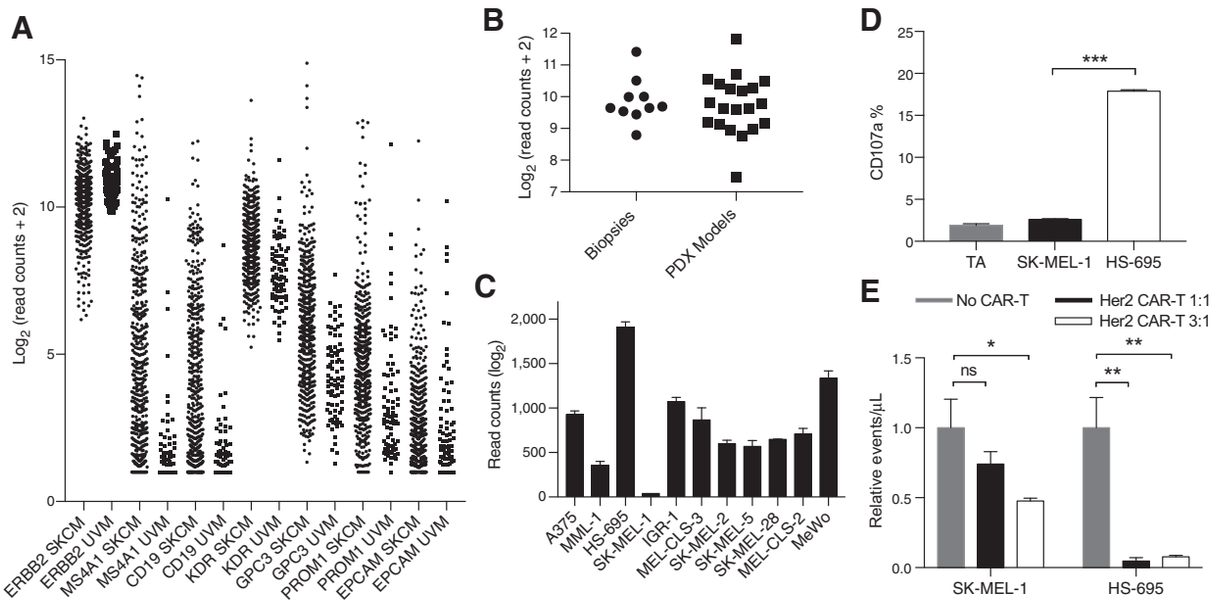

Figure 1.

HER2 is expressed in melanoma. **A,** Expression of *HER2 (ERBB2), CD20 (MS4A1), CD19, VEGFR2 (KDR), glypican-3 (GPC3), CD133 (PROM1)*, and *EpCAM* in skin melanoma (SKCM) and uveal melanoma (UVM) as assessed by RNAseq. Data were obtained from TCGA database using the cbioportal.org website. **B,** Expression of *HER2* in RNAseq data from previously published patient biopsies and PDX models (12). **C,** Expression of *HER2* in previously published RNAseq data from commercial cell lines (13). **D,** Flow cytometry analysis of degranulation marker CD107 in CAR-T cells cultured in the absence (T cells alone; TA) or in the presence of indicated cell lines. **E,** Flow cytometry analysis showing amount of indicated tumor cells after culture in the absence or presence of increasing ratio of CAR-T cells:tumor cells for 48 hours. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, nonsignificant.

antibodies directed against human CD3 (FITC and HIT3a, BioLegend), CD8 (APC and SK1, BioLegend), CD4 (PE and RPA-T4, BD Biosciences), CD107 (APC and H4A3 BD Biosciences), and CD56 (PE-Cy7 and QA17A16, BioLegend).

Tumor growth was monitored using an IVIS Lumina Series II (PerkinElmer) and associated software. CAR-T cells were injected after confirmed increase in bioluminescence (most often 5–10 days after transplantation of tumor cells) and thereafter tumor growth was followed using weekly caliper measurements.

CAR-T cells (2×10^5) were mixed with irradiated (40 Gy) feeder cells (20×10^6), CD3 antibody (clone OKT3, 30 ng/mL, Miltenyi Biotec), and medium containing 50% RPMI1640 and 50% AIM-V (Invitrogen) supplemented with 10% human serum (Sigma-Aldrich) and 6,000 IU/mL IL2 (PeproTech). After 5 days in culture at 37 °C in 5% CO_2 half of the medium was replenished and from day 6 onwards flasks were inspected daily and split when necessary to maintain cell densities around $1–2 \times 10^6$/mL. After 14 days in culture the CAR-T cells were harvested, resuspended in PBS with 300 IU/mL of IL2, and intravenously transplanted to mice (7×10^6 or 20×10^6 cells per mouse in 100 μL).

Statistical analysis

Statistical analysis was performed using GraphPad software, multiple *t* tests (two-stage step-up method of Benjamini, Krieger, and Yekutieli). *P* values are represented as *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

Results

Melanomas express variable levels of HER2 and respond to HER2 CAR-T cells *in vitro*

Clinical trials suggest that around 50% of patients with stage IV cutaneous melanoma may be cured by targeted therapies or immune therapies (4, 10). On the other hand, uveal melanoma, a specific subtype of melanoma arising in the eye, is notoriously resistant to immunotherapy, and because the driver oncogenes *GNAQ* and *GNA11* are not druggable there is no targeted therapy (11). To scrutinize new immune therapies for patients that do not respond to checkpoint blockade, we investigated whether any of the well-known CAR-T targets are expressed in cutaneous or uveal melanoma. Of the most established CAR-T targets (CD19, CD20, HER2, and EpCAM), only HER2 mRNA was expressed at any appreciable levels in most cutaneous melanoma and uveal melanoma datasets from The Cancer Genome Atlas (TCGA) (Fig. 1A). The mRNA expression of HER2 in cutaneous melanoma and uveal melanoma was comparable with that in sarcoma, but less than in breast cancer (Supplementary Fig. S1A), two diseases in which HER2 CAR-T has been extensively investigated. HER2 was also constitutively expressed in an independent dataset from biopsies of patients from Sahlgrenska University Hospital (Gothenburg Sweden; ref. 12) and their corresponding PDXs (Fig. 1B), as well as in commercially available melanoma cell lines (Fig. 1C; ref. 13). The HER2 protein was also detectable by IHC in xenograft models of cutaneous and uveal melanoma (Supplementary Fig. S1B and S1C).

CAR-T cells are dependent on the expression of the target to be able to elicit an antitumor response. To investigate whether HER2 CAR-T could kill the melanoma cells we cultured one cell line with high HER2 expression (HS695; Fig. 1C) and one with low HER2 expression (SK-MEL-1; Fig. 1C) in the presence of increasing doses of allogeneic CAR-T cells directed against HER2. Tumor cell killing was more efficient in HS695 cells than in SK-MEL-1 cells (Fig. 1D-E), suggesting that the CAR-T cells used were on-target.

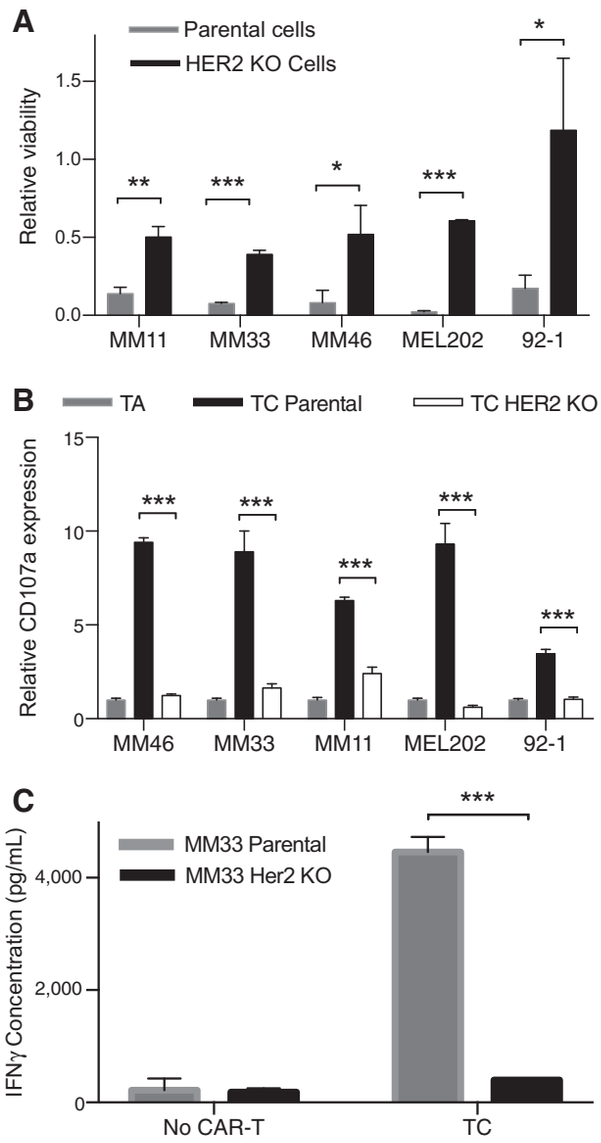

Figure 2.

CRISPR/CAS9-mediated HER2 deletion confers resistance against HER2 CAR-T-cell killing. **A,** Indicated cell lines were transduced with a luciferase and GFP-expressing lentivirus and sorted. Cells were also transfected with a Cas9/gRNA complex directed against HER2. HER2-negative cells were sorted (Supplementary Fig. S2) and both HER2-negative (KO) and parental cells were then cultured in the absence or presence of HER2 CAR-T cells. Viability was measured in a luminometer by adding luciferin to the culture media. **B,** Flow cytometry analysis of degranulation marker CD107 in CAR-T cells cultured in the absence (TA) or in the presence (TC) of indicated HER2-negative or parental cell lines. **C,** CAR-T cells were cultured in the absence or in the presence of parental or HER2-negative MM33 cells. Medium was collected and IFNγ was measured by ELISA. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

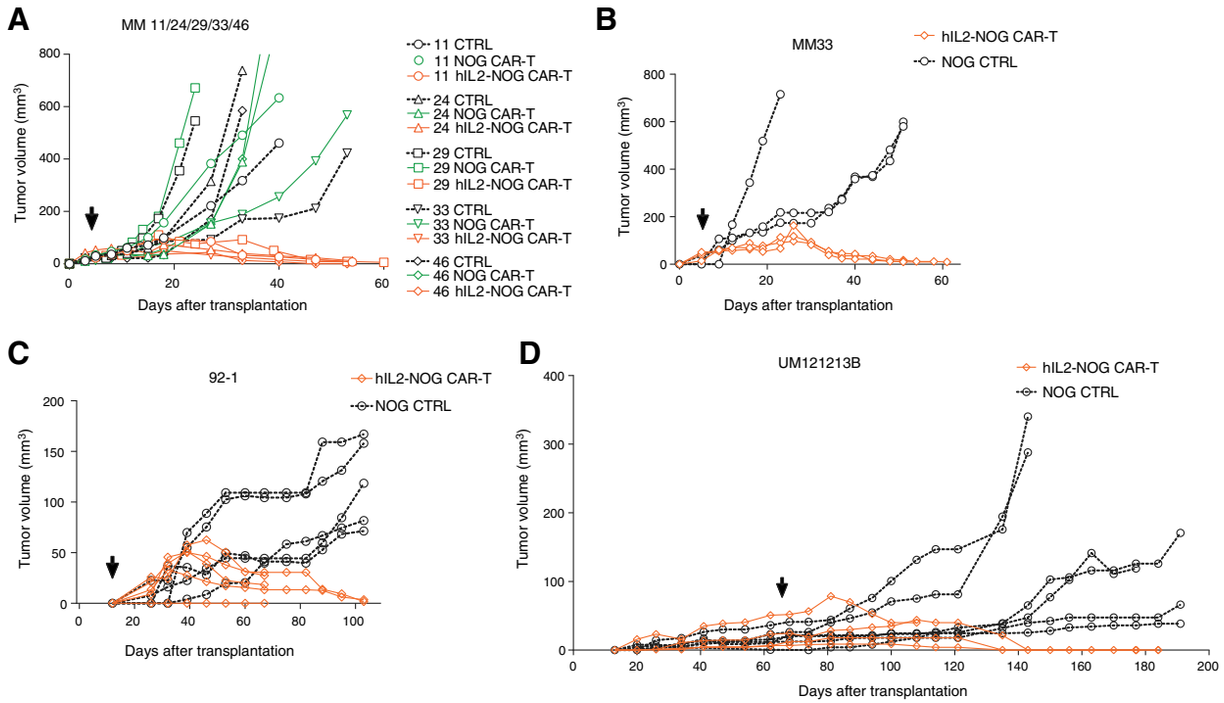

Figure 3.

CAR-T effect *in vivo* is enhanced in IL2 transgenic NOG mice. **A,** Combined data of tumor growth curves from screening of five different patients' PDX models for responsiveness to HER2 CAR-T cells in NOG or in hIL2-NOG mice. Individual patients' PDX growth curves are shown in Supplementary Fig. S2. **B,** Repeat experiment showing tumor growth curves of PDX model MM33 in triplicates. **C** and **D,** Uveal melanoma cell line 92-1 or patient-derived cells from patient UM121213B were injected into NOG or hIL2-NOG mice. At indicated times (arrow), HER2 CAR-T cells were injected and tumor growth was monitored.

HER2 CAR-T cells can kill tumor cells that are nonresponders to TIL therapy

Adoptive T-cell therapy of TILs has shown durable responses in nonrandomized trials (6, 14, 15). However, resistance to immunotherapy can occur owing to many factors including lack of suitable neoantigens, impaired antigen processing, loss of expression of antigen presenting molecules (HLA and B2M), and many other factors (16, 17). To assess whether CAR-T therapy would be an option for patients that do not respond to TIL or anti-PD-1 therapies we made use of cell lines developed from tumors that either regress (MM33) in response to, or are resistant (MM46 and MM11-NR) to killing by their own TILs in mice and/or in patients (9, 15, 18). Treatment with HER2 CAR-T cells resulted in cell death, irrespective whether or not these cells respond to their own TILs (Fig. 2A). Hence, resistance to TIL therapy is not mediated via an inability to respond to T-cell–induced cytolysis in these tumor cell lines. Again, the CAR-T killing was mediated by HER2 because CRISPR/CAS9 deletion rescued the cells from killing by CAR-T cells (Fig. 2A; Supplementary Fig. S2), and reduced degranulation (Fig. 2B) and interferon gamma production (Fig. 2C) by the CAR-T cells. We also investigated whether uveal melanoma cells would be sensitive to HER2 CAR-T cells. To that end commercially available cell lines 92-1 and MEL202 were treated with HER2 CAR-T cells before and after CRISPR/CAS9 deletion of HER2. The uveal melanoma cells were sensitive to HER2 CAR-T cells, in a target-specific manner, suggesting that patients with uveal melanoma could also benefit from CAR-T therapy (Fig. 2A and B; Supplementary Fig. S2).

HER2 CAR-T cells can kill tumor cells that are nonresponders to TIL therapy *in vivo*

To date, no CAR-T therapy against solid cancers has reached a phase III trial. This is partly due to lower frequency and durability of responses in animal models and early trials, as compared with CD19 CAR-T cells in leukemia and lymphoma (19). We have recently shown that ACT of autologous TILs can eradicate human tumors in a novel mouse strain, a human IL2 transgenic NOD/SCID IL2 receptor gamma knockout mice (hIL2-NOG mice), whereas tumors are unaffected by ACT in NOG mice (9, 20). The hIL2-NOG mice are severely immunocompromised but can support viability of human T cells by expressing the cytokine IL2 (9), explaining the persistence and enhanced killing in this strain.

To assess whether HER2 CAR-T can kill melanoma cells *in vivo* we screened five patient-derived cell lines for sensitivity to CAR-T cells when growing in NOG or in hIL2-NOG mice. Adoptive transfer of TILs or CAR-T cells was performed and tumor growth was monitored by caliper measurements. HER2 CAR-T cells caused deep or complete regression of all tumors in hIL2-NOG mice, but not in NOG mice (Fig. 3A; Supplementary Fig. S3). As expected, and published (9), TIL therapy was inefficient in MM46, MM29, and in MM11-NR, which was also the case in the corresponding patients when treated with ACT (15, 18). The one mouse/one therapy/one dose (1+1+1) approach used here is established for screening targeted therapies in PDX models (21) but to show robustness also for CAR-T therapy we repeated the experiment in a M33F8 xenograft, showing identical results

(Fig. 3B). Moreover, the CAR-T cells could also cause tumor rejection in two models of uveal melanoma; one cell line xenograft made with the commercially available cell line 92-1 (Fig. 3C), and one PDX model of metastatic uveal melanoma from Sahlgrenska University Hospital (Gothenburg Sweden; Fig. 3D), which carried an oncogenic mutation of GNA11 and exhibited histopathologic similarity to the original biopsy (Supplementary Fig. S4). The therapeutic effects were associated with presence of $CD3^+$ T cells in regressing tumors (Supplementary Fig. S5A) and in blood of hIL2-NOG mice (Supplementary Fig. S5B-C). No evidence of expansion of CD3–$CD56^+$ natural killer (NK) cells was evident in the cells used for injection or in the mice but CD56 was expressed on a fraction of T cells, likely representing NK T cells or cytokine-induced killer cells.

Discussion

Here we have gathered preclinical evidence that HER2 CAR-T cells can eradicate cutaneous and uveal melanoma *in vitro* and in mice. It is conceivable that this CAR-T target has been missed by the melanoma researchers. Of particular interest is the observation that CAR-T cells can kill uveal melanoma, a disease that neither have any options for targeted therapy, they are *BRAF* wild-type, or for immunotherapy because only few patients responds to checkpoint blockade (11). We also demonstrate that CAR-T cells can exhibit activity against melanoma cells resistant to other types of T-cell immunotherapy. The fact that HER2 CAR-T cells are known to have a reasonable safety (22) should help in clinical translation and could result in a novel treatment option for patients with skin and uveal melanoma.

Another important finding is that CAR-T cells were more efficacious in IL2 transgenic mice. This positions the hIL2-NOG mouse as the optimal humanized mouse model to test novel CAR-T cells against solid tumors. The IL2 dependency likely also means that one of the reasons why CAR-T cells have limited clinical efficacy against solid tumors to date is that exhaustion and suppressive immune cells cause a reduction in IL2 signaling in the tumor microenvironment (23). Our results are therefore in favor of those attempts to create CAR-T cells which are self-sufficient in IL2 signaling (24) and unresponsive to exhaustion signals (25).

Disclosure of Potential Conflicts of Interest

R.O. Bagge has received speakers bureau honoraria from Roche and is a consultant/advisory board member for MSD, BMS, and Amgen. L. Ny reports receiving other commercial research support from Merck Inc./MSD Sweden and Syndax Pharmaceuticals, has received speakers bureau honoraria from AstraZeneca, Bristol Myers Squibb, and MSD Sweden, and is a consultant/advisory board member for Bristol Myers Squibb, MSD Sweden, Novartis, and Pierre Fabre. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.M.V. Forsberg, M.F. Lindberg, H. Jespersen, S. Alsén, R.O. Bagge, M. Donia, O. Nilsson, L. Ny

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E.M.V. Forsberg, M.F. Lindberg, H. Jespersen, S. Alsén, O. Nilsson, J.A. Nilsson

Writing, review, and/or revision of the manuscript: E.M.V. Forsberg, M.F. Lindberg, H. Jespersen, S. Alsén, R.O. Bagge, I.M. Svane, O. Nilsson, L. Ny, L.M. Nilsson, J.A. Nilsson

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.O. Bagge, L.M. Nilsson

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References

1. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711–23.
2. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320–30.
3. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med 2015;372:2521–32.
4. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med 2017;377:1345–56.
5. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science 2018;359:1350–5.
6. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med 1988;319:1676–80.
7. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov 2013;3:388–98.
8. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med 2018;378:439–48.
9. Jespersen H, Lindberg MF, Donia M, Söderberg EMV, Andersen R, Keller U, et al. Clinical responses to adoptive T-cell transfer can be modeled in an autologous immune-humanized mouse model. Nat Commun 2017;8:707.
10. Long GV, Eroglu Z, Infante J, Patel S, Daud A, Johnson DB, et al. Long-term outcomes in patients with BRAF V600-mutant metastatic melanoma who received dabrafenib combined with trametinib. J Clin Oncol 2018;36:667–73.
11. Yang J, Manson DK, Marr BP, Carvajal RD. Treatment of uveal melanoma: where are we now? Ther Adv Med Oncol 2018;10:1758834018757175.
12. Einarsdottir BO, Bagge RO, Bhadury J, Jespersen H, Mattsson J, Nilsson LM, et al. Melanoma patient-derived xenografts accurately model the disease and develop fast enough to guide treatment decisions. Oncotarget 2014;5:9609–18.
13. Bhadury J, Einarsdottir BO, Podraza A, Bagge R, Stierner U, Ny L, et al. Hypoxia-regulated gene expression explains differences between melanoma cell line-derived xenografts and patient-derived xenografts. Oncotarget 2016;7:23801–11.
14. Besser MJ, Shapira-Frommer R, Itzhaki O, Treves AJ, Zippel DB, Levy D, et al. Adoptive transfer of tumor-infiltrating lymphocytes in patients with metastatic melanoma: intent-to-treat analysis and efficacy after failure to prior immunotherapies. Clin Cancer Res 2013;19:4792–800.

15. Andersen R, Donia M, Ellebaek E, Borch TH, Kongsted P, Iversen TZ, et al. Long-lasting complete responses in patients with metastatic melanoma after adoptive cell therapy with tumor-infiltrating lymphocytes and an attenuated IL2 regimen. Clin Cancer Res 2016;22:3734–45.
16. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015;348:69–74.
17. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations associated with acquired resistance to PD-1 blockade in melanoma. N Engl J Med 2016;375:819–29.
18. Donia M, Harbst K, van Buuren M, Kvistborg P, Lindberg MF, Andersen R, et al. Acquired immune resistance follows complete tumor regression without loss of target antigens or IFNγ signaling. Cancer Res 2017;77:4562–6.
19. D'Aloia M, Zizzari I, Sacchetti B, Pierelli L, Alimandi M. CAR-T cells: the long and winding road to solid tumors. Cell Death Dis 2018;9:282.
20. Einarsdottir BO, Karlsson J, Söderberg EMV, Lindberg MF, Funck-Brentano E, Jespersen H, et al. A patient-derived xenograft pre-clinical trial reveals treatment responses and a resistance mechanism to karonudib in metastatic melanoma. Cell Death Dis 2018;9:810.
21. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med 2015;21:1318–25.
22. Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C, et al. Human epidermal growth factor receptor 2 (HER2) –specific chimeric antigen receptor–modified T cells for the immunotherapy of HER2-positive sarcoma. J Clin Oncol 2015;33:1688–96.
23. Carter L, Fouser LA, Jussif J, Fitz L, Deng B, Wood CR, et al. PD-1:PD-L inhibitory pathway affects both $CD4^+$ and $CD8^+$ T cells and is overcome by IL-2. Eur J Immunol 2002;32:634–43.
24. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang C-H, Saso K, et al. A novel chimeric antigen receptor containing a JAK–STAT signaling domain mediates superior antitumor effects. Nat Med 2018;24:352.
25. Ren J, Liu X, Fang C, Jiang S, June CH, Zhao Y. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clin Cancer Res 2017;23:2255–66.