our group could previously demonstrate that arming CAR T cells with C-C-motive-receptor 8 for improved tumor-directed migration along the C-C-chemokine ligand 1 - CCR8 axis and a dominant-negative receptor against TGF-β for resistance to suppression enable activity in pancreatic cancer models. The value of this approach for other entities was however unclear. We now investigated the potential of this combination for treatment of HER2-positive cancer models in conjunction with a HER2-targeted CAR.

Materials and Methods

Primary murine and human T cells were isolated and activated. T cells were retrovirally transduced. Phenotype, activation, exhaustion and proliferation were monitored in vitro. Cytokine production was assessed with ELISA. In vivo, survival and tumor growth of mice that were subcutaneously injected with tumor cells and treated with CAR T cells carrying either CCR8, DNR or both receptors were measured. To look at chemokine expression in tumor material, mRNA was isolated from tumor material and RT-qPCR was performed.

Results

We found that expression of CCR8 can redirect CAR T cells to the tumor and a DNR can prevent immunosuppression of CAR T cells in the tumor microenvironment. The improved functionality of CAR-CCR8-DNR T cells compared to CAR T cells against the HER2 antigen could be demonstrated in vitro and in vivo in human HER2+ tumor models.

Conclusions

Equipping CAR T cells with CCR8 and DNR emerges as a strategy not only limited to certain antigens, but as a potential universal approach to render cellular therapies more effective. The modularity of this concept promises further preclinical and perhaps clinical development to improve personalized immunotherapy.

Disclosure Information

T.J. Strzalkowski: None. B.L. Cadilha: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. I. Dalloiu: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. K. Manske: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. S. Endres: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; TCR2 Inc, Bio-M, Munich, Germany. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; Else Kröner-Fresenius Stiftung, Paul-Martini-Stiftung. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Carina Biotech Ltd, Mawson Lakes, Australia. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; TCR2, Cambridge, MA, USA. F. Consultant/Advisory Board; Significant; Gilde Healthcare, Utrecht, Netherlands. S. Kobold: A. Employment (full or part-time); Significant; University Hospital, LMU Munich. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; TCR2 Inc, Arcus. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; Els Kröner-Fresenius Stiftung, Paul-Martini-Stiftung. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Carina Biotech Ltd, Mawson Lakes, Australia. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; TCR2, Cambridge, MA, USA. F. Consultant/Advisory Board; Significant; Tabby therapeutic ltd. F. Consultant/Advisory Board; Significant; TCR2 Inc, Novartis.

BISPECIFIC ANTIBODIES ENABLE SYNTHETIC AGONISTIC RECEPTOR T CELL THERAPY IN MELANOMA

Background

Immunotherapies, like immune checkpoint inhibition and tumor infiltrating lymphocytes, have had remarkable success in treating melanoma. However, many patients do still not respond or relapse with therapy-resistant disease. To overcome said limitations, we propose a controlled adoptive cell therapy approach, where T cells are armed with EGFRvIII synthetic agonistic receptors (E3 SAR) that are selectively activated by a cross-linking bispecific antibody (BiAb) specific for both SAR T cell and melanoma-associated antigens.

Materials and Methods

Murine as well as human SAR constructs were generated and T cells were retrovirally transduced to stably express the SAR constructs. We validated our approach in murine, human and patient-derived cancer models expressing the melanoma-associated target antigens TPR1 and MCSP. SAR T cells were functionally characterised by proving specific activation and proliferation of SAR T cells, as well as their tumor-directed cytotoxicity, in vitro and in vivo.

Results

Both on a mRNA and protein level, MCSP and TPR1 were shown to be differentially expressed in treatment-naïve as well as treatment-resistant melanoma patients compared to samples from healthy donors. Crosslinking anti-TPR1 x anti-E3 and anti-MCSP x anti-E3 BiAb mediated conditional antigen-dependent activation, proliferation of SAR-T cells and lead to tumor cell lysis in all models tested. In vivo, anti-tumoral activity and tumor-free survival was mediated by the co-administration of SAR T cells and BiAb in a syngeneic tumor model and was further confirmed in several xenograft models.

Conclusions

Here, we apply the SAR x BiAb approach in an effort to deliver specific and conditional activation of SAR transduced T cells, and targeted tumor cell lysis in melanoma models. The modularity of our approach is key for targeting melanoma and is essential towards personalised immunotherapies addressing cancer heterogeneity. Due to variations of antigen expression in primary melanoma tissues, we propose that a dual-targeting approach, either simultaneous or sequential, could mitigate issues of heterogeneity and deliver therapeutic benefit to patients.

Disclosure Information

M. Bennebaek: A. Employment (full or part-time); Significant; Klinikum der Universität München. F. Märl: A. Employment (full or part-time); Significant; Klinikum der Universität München. J. Keyl: None. B. Cadilha: A. Employment (full or part-time); Significant; Klinikum der Universität München. C. Karches: A. Employment (full or part-time); Significant; Klinikum der Universität München. H. Obeck: None. M. Schwerdtfeger: A. Employment (full or part-time); Significant;
Klinikum der Universität München. D. Briukhovetskaya: A. Employment (full or part-time); Significant; Klinikum der Universität München. J. Jobst: None. P.J. Müller: None. M. Seevert: None. R. Grünebier: None. M. Thomas: A. Employment (full or part-time); Significant; Helmholtz München. C. Marr: A. Employment (full or part-time); Significant; Helmholtz München. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; European Research Council. M. Levesque: A. Employment (full or part-time); Significant; University Hospital Zurich. M. Hepp: A. Employment (full or part-time); Significant; Universitätsklinikum Erlangen. S. Endres: A. Employment (full or part-time); Significant; Klinikum der Universität München. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Elite Network of Bavaria, LMU Munich’s Institutional Strategy LMUexcellent. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Patents in the field of immuno-oncology. C. Klein: A. Employment (full or part-time); Significant; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Stocks and patents with Roche. S. Kobold: A. Employment (full or part-time); Significant; Klinikum der Universität München. B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Volkswagen Foundation, European Research Council, Hector Foundation, Elite Network of Bavaria, Melanoma Research Alliance, Else Kröner-Fresenius-Stiftung, German Cancer Aid, Ernst-Jung-Stiftung, LMU Munich’s Institutional Strategy LMUexcellent, Bundesministerium für Bildung und Forschung, European Research Council Grant, Fritz-Binder Foundation, José-Carreras Foundation. C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; German Research Foundation. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Significant; TITCR2 Inc, Novartis, BMS, GSK. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Patents in the field of immuno-oncology.

**CD122-DIRECTED IL-2/ANTI-IL-2 COMPLEXES ENHANCE ABSOCAL RESPONSES TO RADIATION COMBINED WITH ANTI-PD-1**

CD122-Directed IL-2/anti-IL-2 complexes enhance abscopal responses to radiation combined with anti-PD-1.

Background Early clinical trials have provided evidence for RT-induced systemic effects in conjunction with αPD-1 or IL-2 in metastatic patients, but strong abscopal responses are clinically rare. Dual combinations of αPD-1 with more effective and less toxic IL-2 derivatives, e.g., CD122-directed pegylated IL-2, are also currently under investigation. Whether a combination of RT, αPD-1, and CD122-directed IL-2/anti-IL-2 complexes (IL-2c) can increase abscopal effects against established non-irradiated tumors is unknown. Also, in-depth analyses of the differentiation of tumor-specific CD8+ T cells have not yet been reported for αPD-1/IL-2c in combination with RT. We investigated how adding IL-2c to hRT/αPD-1 affects tumor-specific CD8+ T cell differentiation in mouse tumor models and the potential of this triple combination to enhance the abscopal effect compared to the respective single treatments.

Materials and Methods Mice bearing bilateral tumors were treated with two fractions of 8 Gy (C51 colon carcinoma model) or 12 Gy (B16 melanoma model); αPD1 was given weekly; IL-2c was given for five consecutive days. CD8 T cell-depleting and CXCR3-blocking antibodies were used to determine if the therapeutic effects depend on CD8+ and CXCR3+ T cells. Differentiation stages of tumor-specific CD8+ T cells in tumor-draining lymph nodes, spleen, blood, and tumors were determined by flow cytometry using MHC-I tetramers and various antibodies.

Results The abscopal effect was significantly stronger in triple-treated mice compared to mice treated with RT/αPD-1 (C51 model: P < 0.01; B16 model: P < 0.05), RT/IL-2c (C51 model: P < 0.01; B16 model: P < 0.001) or αPD1/IL-2c (C51 model: P < 0.0001, B16 model: P < 0.01). Moreover, triple therapy improved survival and resulted in complete cures of 3/12 mice in the C51 model and 2/12 mice in the B16 model. These anti-tumor effects were associated with dramatic expansion of tumor-specific CD8+ T cells. Undifferentiated stem-like and effector-like but not terminally differentiated exhausted cells particularly strongly increased. Moreover, IL-2c induced CXCR3 expression on tumor-specific CD8+ T cells. Both CD8+ (C51 model: P < 0.0001; B16 model: P < 0.01) and CXCR3+ (C51 model: P < 0.0001) T cells were crucial for the RT-induced abscopal effect upon RT/αPD-1/IL-2c treatment.

Conclusions RT/αPD-1/IL-2c triple treatment resulted in superior local and systemic expansion of tumor-specific CD8+ T cells with stem- and effector-like phenotypes. Also, IL-2c strongly increased CXCR3+ CD8+ T cells that were associated with pronounced abscopal responses in models with an established metastasis resistant to αPD-1/IL-2c and only transiently responding to RT/αPD-1 or RT/IL-2c. Therefore, such triple combinations appear promising for clinical evaluation in metastatic patients.


**SECONDARY RESISTANCE TO IMMUNOTHERAPY IS ASSOCIATED WITH DEATH AND DE-DIFFERENTIATION OF ACTIVATED T CELLS**

C. Qing; E. Ghiorani; K. Foster; M. Amann; I. Uddin; G. Beattie; I. Solomon; F. Arce-Vargas; K. Peggs; S. Quezada. Cancer Institute, University College London, London, UK; Roche Innovation Center Zurich, Zurich, Switzerland

Background Immunotherapies have transformed the care of patients with multiple tumor types, but the majority who respond eventually progress after a period of stabilization. The reasons for this are not well known. We set out to explore mechanisms of immunotherapy failure in this setting, using a murine model of melanoma treated with a regulatory T cell (Treg)-depleting antibody combined with a cancer cell vaccine.

Materials and Methods C57BL/6 mice were injected subcutaneously with B16 cells. Treatment with a mouse IgG2a depleting αCD25 antibody was given on day 5, followed by a B16