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Engineering hematopoietic stem cells to create melanoma specific CTL

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Using a humanized mouse model, we developed melanoma specific CD8 T cells from genetically modified human hematopoietic stem cells (hHSC). The transgenic T cells were functional both in vivo and ex vivo, effectively limiting and clearing tumor growth. Finally, the transduced hHSC stably populated the bone marrow.

The human immune system is a formidable weapon against cancer and immune-based therapies have been used to combat this chronic disease. Such therapies include the adoptive transfer of tumor specific autologous T cells or the introduction of tumor specific T-cell receptors (TcR) via lentiviral vectors into peripheral blood T cells, which has been used with some success in melanoma.[1,2] The introduction of exogenous TcR into mature T cells while promising harbors the risk of generating autoreactive clones[3] and results in T-cell exhaustion due to the ex vivo manipulation required for the transduction of these cells.[4] These limitations can be addressed by introducing the exogenous TcR into hematopoietic progenitor cells, which would then differentiate into mature tumor specific T cells. Such an approach allows for the proper maturation and selection of the transgenic T cells and, due to the self-renewing nature of stem cells, can result in a long-term and stable source of tumor specific T cells.[5]

The recent advancements in humanized mouse models have made it possible to address this approach in vivo. For our studies, we used an adaptation of the bone marrow/liver/thymus (BLT) humanized mouse model.[6] In this model, fetal human thymus combined with fetal liver is transplanted under the kidney capsule of the immunodeficient NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mouse. This is followed by sublethal irradiation and transplantation of autologous human hematopoietic stem cells (hHSC). The outcome is full reconstitution of the immune system with human cells.[7,8] Furthermore, the hHSC can be genetically modified and give rise to engineered functional mature lymphocytes (modified BLT).[8] Thus, this model was the most appropriate system to examine in vivo both the generation and the functionality of melanoma-specific transgenic CD8 T cells (CTL) against human tumor challenge.

To this end we used the modified BLT to assess whether we can in vivo generate and assess MART-1 specific CTL. We constructed a lentiviral vector encoding the F5 MART-1 TcR, an HLA-A*0201-restricted melanoma-specific TcR, and optimized herpes simplex virus 1 thymidine kinase (sr39tk) gene which serves both as a PET reporter and a suicide gene. The vector was introduced into HLA-A*0201$^+$ hHSC, which were combined with autologous fetal thymic tissue to make the thy/liv implant. A second fraction of the transduced hHSC was then used for transplantation 3 weeks later. Approximately 4–6 weeks after hHSC transplantation, we assessed reconstitution of MART-1 transgenic CTL in the murine circulation. We detected high levels of mature transgenic CTL that maintained a naïve phenotype (based on expression of CD62L and CD27). The MART TcR was rarely detected on CD4 T cells suggesting that the proper T-cell selection and lineage commitment took place. To confirm that the selection was taking place in the thy/liv implant and not the mouse thymus, we tested the above approach on an HLA-A*0201$^-$ background. We did not detect any CD8 or CD4 T cell expressing the TcR in the periphery.

Following reconstitution with the transgenic CTL, the chimeric mice were challenged with the human melanoma tumor lines M202, an HLA-A*0201$^+$MART$^+$ cell line that could serve as a target for the transgenic CTL, and M207, an HLA-A*0201$^-$MART$^+$ line that could not. The tumors were implanted on the left (M202) and right (M207) mouse shoulder. Tumor regression was assessed by both physical measurements and PET imaging (**Fig. 1**). PET imaging proved particularly beneficial and quite effective in assessing the efficacy of the transgenic CTL. The physical measurements, while demonstrating decreasing tumor sizes, were masked in some occasions by tissue scarring or "dead space" due to tissue necrosis caused by the anti-tumor immune response resulting in the appearance of false positive tumor growth. Thus, at the endpoint of our experiment (about 12 weeks after hHSC transplantation), we used PET imaging for [^{18}F]FDG uptake to measure tumor metabolic activity by glucose uptake. The data revealed that in 7/9 mice we had targeting of the M202 tumor with

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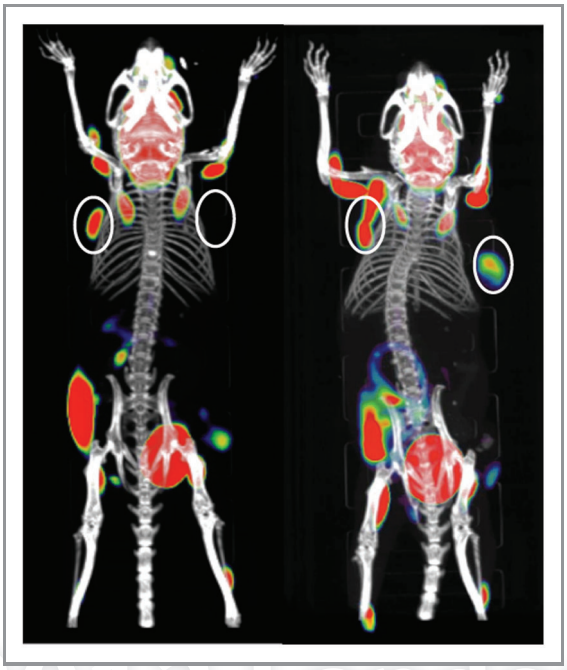

Figure 1. MART-1 specific CTL limit and clear growth of matched melanoma tumors. In vivo PET imaging was performed to assess the levels of FDG uptake by the M202 and M207 tumors. The circles indicate the location of the M202 (right) and M207 (left) tumors.

4/9 mice showing complete tumor clearance (**Fig. 1**). There was some limited regression in the M207 control tumors potentially due to bystander activation of an allogeneic response. Lastly, we detected increased infiltration of MART CTLs into the M202 tumors.

Further ex vivo analysis of the transgenic CTLs revealed that these cells had undergone the appropriate antigen induced maturation. Splenocytes from the treated mice effectively killed the M202 targets in a CTL killing assay in the absence of any additional activation. When compared with cells from control mice (mice receiving non transduced hHSC), which were predominantly naive, the transgenic MART CTLs from spleen were comprised of naïve ($CD45RA^+CCR7^+$), central ($CD45RA^-CCR7^+$), effector ($CD45RA^-CCR7^-$) memory and terminally differentiated ($CD45RA^+CCR7^-$) cells. Finally, our data showed a correlation between tumor metabolic activity and levels of reconstitution or ex vivo cytolytic activity.

As stated earlier, this approach would be advantageous due to the longevity of hHSC. This would result in the continuous production of MART-1 specific CTL, thus maintaining a high precursor frequency to control tumor growth and/or relapse. We collected bone marrow cells from the treated and control chimeras and detected the presence of the lentiviral vector (by real time PCR) and the expression of the sr39tk reporter (by flow cytometry).

In summary, the impact of our studies is 2-fold. First, we demonstrate as a proof of principle that functional tumor specific CTLs can be generated from genetically modified human hematopoietic progenitors. Second, we have developed a model that can be utilized to explore a wide span of alternative or combination treatments against cancer and chronic diseases.[9]

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