Monocytes/macrophages are required for both optimal anti-leukaemia efficacy and the cytokine release syndrome by CAR-T cells

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Background: Chimeric antigen-receptor (CAR)-engineered T cells promise to cure chronic and acute leukaemias relapsed after or refractory to standard treatments. Before this promise is fulfilled, however, a number of issues need to be solved, namely how to manage associated toxicities (e.g., the cytokine release syndrome, CRS; depletion of target antigen-expressing lineages) and how to circumvent secondary resistance mechanisms (e.g., those due to target-antigen loss or leukaemic lineage switch). Unfortunately, all these issues cannot be addressed pre-clinically in currently available NSG mouse models, because they lack human hematopoiesis and develop xenograft-versus-host disease (X-GVHD). Methods: We have developed an innovative xenotolerant model by transplanting human hematopoietic stem cells (HSCs) intraliver in newborn NSG mice triple transgenic for human stem cell factor, GM-SF and IL-3 (SGM3). Differently from “classical” NSG, SGM3 mice reconstituted high levels of human T cells, which, once transferred in secondary recipients, persisted up to 200d without causing X-GVHD. We therefore designed secondary transfer experiments in leukaemic and/or HSC-humanised SGM-3 mice for studying the determinants of CAR-T cell efficacy and associated toxicities in the absence of confounding xenoreactivity. Results: SGM3-derived T cells were transduced ex vivo with either a CD19 or a CD44v6 CAR after activation with CD3/CD28-beads and IL-7/IL-15. Once transferred in secondary recipients previously engrafted with a CD19+/CD44v6+ leukaemic semi-cell line, CD19 or CD44v6 CAR-T cells equally mediated rapid tumour clearance, both in low and high tumour-burden settings, in the absence of malaise or elevated human IL-6 levels in vivo. At later time points (after 100d), however, approximately 50% of responding mice relapsed despite significant CAR-T cell persistence in vivo (>50 cells per microl). A significant fraction of leukaemia relapses were characterized by post-transcriptional down-regulation of CD44v6 expression or CD19 loss, respectively. Conversely, secondary transfer of SGM3-derived CAR-T cells in leukaemic SGM3 mice that had been previously humanised with HSCs resulted in the development of the CRS (high fevers, elevated IL-6, TNF-alpha and serum amyloid A levels), resulting in 30% lethality. Strikingly, mice recovering from the CRS benefited from durable leukaemia remissions, yet experienced long-lasting CD19+ B-cell or CD44v6+ monocyte aplatias. Interestingly, in this model, tocilizumab administration at the time of either CD19 or CD44v6 CAR-T cell infusion efficiently prevented the CRS, but did not interfere with anti-leukaemia effects in the long-term. Conversely, depleting monocytes/macrophages by prophylactic CD44v6 CAR-T cells inhibited CRS development, but also resulted in significantly worse leukaemia-free survival (at 250d, 0% vs 80%, P<0.0001). Conclusions: A number of lessons can be learned from this innovative xenotolerant mouse model of CAR-T cell immunotherapy: i) monocytes/macrophages are required for both optimal anti-leukaemia efficacy and the CRS; ii) prophylactic tocilizumab administration does not interfere with anti-leukaemia efficacy, iii) monocyte aplasia induced by CD44v6 CAR-T cells may ameliorate CRS toxicity. These results have been included in the regulatory package for a phase I/IIa clinical trial in relapsed refractory acute myeloid leukaemia expected to start in 2017.

Disclosure of conflict of interest

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