HemaSphere
Powered by EHA

EHA EUROPEAN HEMATOLOGY ASSOCIATION

Cellular and gene therapy - Section 4

Invariant NKT cells as a platform for CAR immunotherapy and prevention of acute Graft-versus-Host Disease

Anastasios Karadimitris[1,2], Carme Ripoll-Fiol[1], Jose Costa Guerra[1]

[1]Centre for Haematology, Imperial College London, London, United Kingdom; [2]Department of Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

Take home messages

- Pre-clinical and clinical evidence supports a critical role of donor iNKT cells in protection from aGVHD and their potential for its prevention.
- iNKT cells provide optimal platform for chimeric antigen receptor-based immunotherapy of blood cancers.
- iNKT cell-based, 'off-the-shelf' immunotherapy could be sourced from allogeneic, healthy donors without risk of aGVHD.

Introduction

Invariant NKT (iNKT)-cells are a rare (<0.1% of blood T-cells), evolutionarily conserved subset of TCRαβ T-cells sharing features of innate and adaptive immune responses.[1,2] In humans they are characterised by an invariant TCRVα24Jα18 chain nearly always pairing with a diverse TCRVβ11 chain. iNKT-cells are restricted by CD1d, a non-polymorphic, glycolipid-presenting HLA class I-like molecule[3,4] and their development depends on CD1d-expressing double positive thymocytes.[5]

iNKT-cells have a memory effector phenotype with pronounced ability to migrate and home to extra-lymphoid tissues.[6] CD4+ and CD4- iNKT-cell subsets are functionally distinct (eg, Th1 vs Th1/Th2 profile, primarily cytotoxic vs immunoregulatory and differential migratory/homing profile respectively).[7,8] iNKT-cells modulate a variety of immune responses[1,9] that include enhancement (usually) of anti-tumour and anti-pathogen responses and protection from auto-immunity and allo-reactivity, in particular acute graft-versus-host disease (aGVHD).*[10,11] As such, iNKT-cell-based immunotherapy can be sourced from 3rd party donors as 'an-off-the shelf' treatment.

Current state-of-the-art

iNKT cells for prevention of aGVHD

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a curative therapeutic approach for hematological malignancies. However, its wider applicability is prevented by aGVHD, the donor T-cell-mediated alloreactive process responsible for much of the morbidity and mortality associated with allo-SCT.[12,13]

Extensive pre-clinical and clinical observational studies show that donor iNKT cells can prevent aGVHD without increasing the risk of disease relapse. Adoptive transfer of donor $CD4^+$ iNKT-cells ($CD4^-$ cells were not tested), either without manipulation[14,*15,16,17] or following in vitro expansion in the presence of alpha-galactosylceramide (αGalCer),[18] a glycolipid that selectively and powerfully activates iNKT-cells, prevents or alleviates established experimental aGVHD in a MHC mismatched setting. This protective effect is mediated through Th2 polarisation of alloreactive T-cells and expansion of donor regulatory T-cells (Tregs).[14,*15,16,17] Indeed, adoptively transferred donor iNKT-cells are at least 10 times more potent than Tregs in protecting mice from lethal aGVHD without compromising the graft-versus-leukaemia effect.[14,*15,16,17] In addition, adoptively transferred in vitro αGalCer -expanded human iNKT-cells ameliorate xenogeneic aGVHD[19] and improve survival. This protective effect is mediated by $CD4^-$ but not $CD4^+$ human iNKT-cells and involves depletion of murine dendritic cells (DC) in vivo, reduction of T-cell activation and induction of their Th2/Th17 polarisation in vivo.[19] These findings are in line with previous work showing that human iNKT-cells can be activated by allogeneic DC in a CD1d- and activating KIR-dependent manner followed by lysis of the DC.[20]

The protective impact of iNKT-cells in the context of allo-SCT was further highlighted in clinical observational studies. Upon

All three authors contributed to the writing of the manuscript.

Work in the authors' laboratory is supported by Bloodwise and the Imperial NIHR Biomedical Research Centre.

The authors acknowledge research funding from Bloodwise, The Wellcome Trust, Imperial NIHR Biomedical Research Centre, Imperial Confidence in Concept and Imperial Experimental Cancer Medicine Centre.

The authors declare no conflicts of interest.

HemaSphere (2019) 3:S2

Received: 31 January 2019 / Received in final form: 19 March 2019 / Accepted: 19 March 2019

Citation: Karadimitris A, Ripoll-Fiol C, Guerra JC. Invariant NKT cells as a platform for CAR immunotherapy and prevention of acute graft-versus-host disease. *HemaSphere*, 2019;3:S2. http://dx.doi.org/10.1097/HS9.0000000000000220

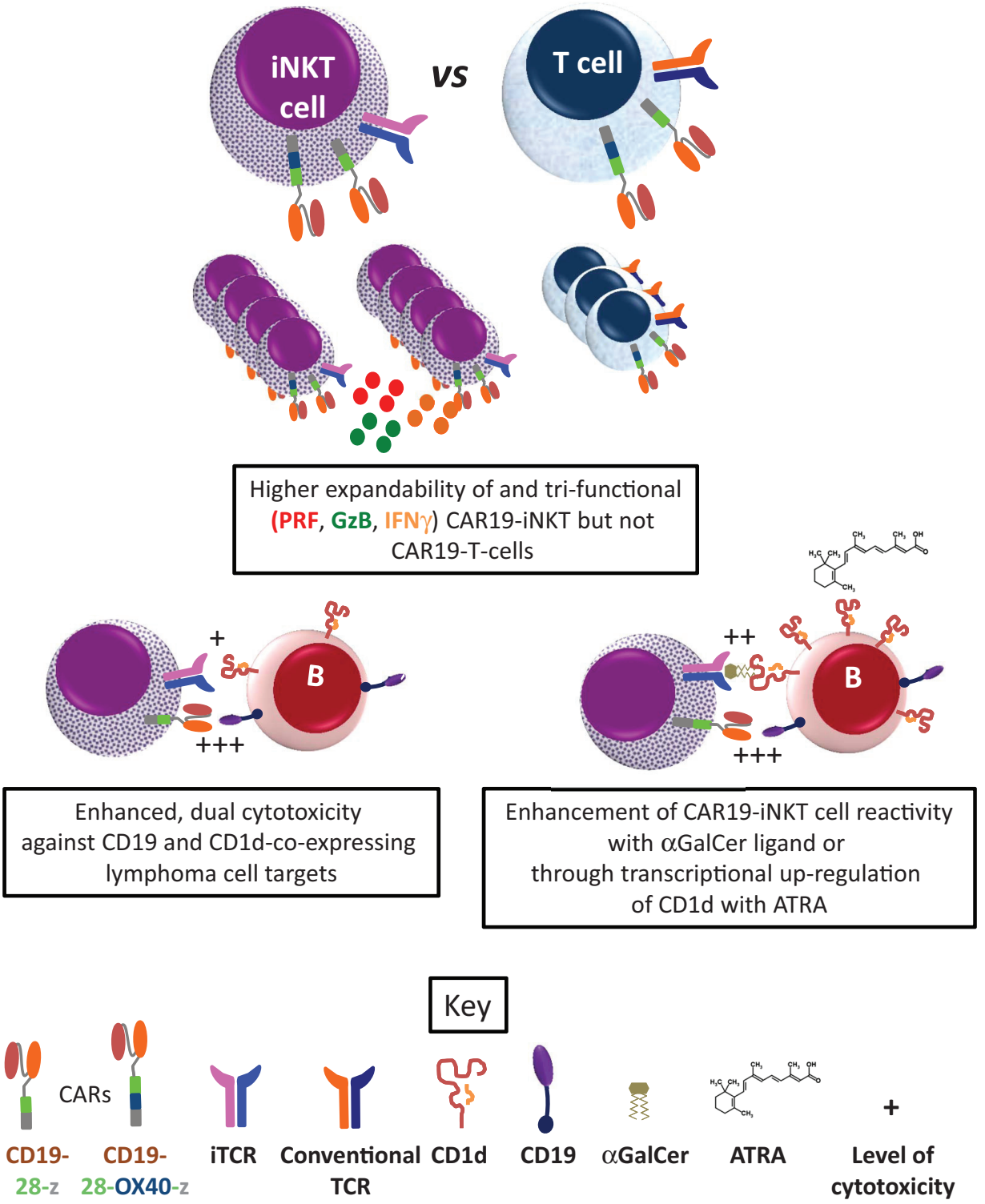

Figure 1. Potential advantages of CAR-iNKT cells over CAR19-T cells for the treatment of CD1d-expressing B lineage malignancies. The higher proliferative and cytotoxic potential of CAR19-iNKT cells against lymphoma cells, their ability to secrete higher levels of anti-tumour molecules and the prospect of enhancing CAR-iNKT cell-based immunotherapy with ATRA and αGalCer are highlighted.

multivariate analysis, amongst several clinical and biological parameters including various immune cell subsets, donor graft $CD4^-$ iNKT-cell dose ($> 0.03 \times 10^6$/kg) was associated with a 4.27-fold reduction in the relative risk of clinically significant aGVHD in a T-cell replete allo-SCT setting.[*10] $CD4^+$ iNKT-cell dose had a significant impact on univariate analysis and a trend towards significance on multivariate analysis,[*10] suggesting that as shown in murine pre-clinical models of aGVHD, there might still be a role for this subset in protection from clinical aGVHD. In line with these findings, early recovery of iNKT-cells post T-cell-

depleted allo-SCT is associated with reduced risk of aGVHD while a higher $CD4^-$ iNKT-cell dose and $CD4^-$ iNKT/CD3 T-cell ratio in the graft were associated with protection from aGVHD.[21,*22]

To-date, no clinical trials involving adoptive transfer of donor iNKT-cells to allo-SCT recipients have been reported and therefore adverse effects cannot be predicted. However, in a study involving infusion of up to 10^9 in vitro expanded autologous iNKT-cells in cancer patients only grade 2 toxicity was observed.[23]

iNKT-cells as a platform for CAR immunotherapy

Chimeric antigen receptor (CAR)-T cell immunotherapy for B lineage malignancies has yielded very promising therapeutic results.[24,25] Several aspects of CAR immunotherapy are under further optimization including the development of technologies which would allow sourcing of CAR-T cells from healthy donors for 'off-the-shelf' use. For this purpose and in order to prevent aGVHD mediated by allogeneic T-cells, the endogenous TCR is deleted by means of gene editing.[26,27] Since allogeneic iNKT-cells do not cause aGVHD they would be an ideal platform for 'off-the-shelf' CAR immunotherapy without need for deletion of their TCR.

Metelitsa et al provided the first evidence that CAR engineering of iNKT-cells and their clinical scale expansion was feasible.[*28,*29] Indeed anti-GD2 and -CD19 human CAR-iNKT-cells were reactive against experimental neuroblastoma and $CD19^+$ lymphoma, respectively, without evidence of xenogeneic aGVHD. However, in both xenograft tumor models, in vivo anti-tumor activity of CAR-iNKT-cells required either repeated CAR-iNKT cell dosing or IL-2 administration.[*28,*29]

More recent work demonstrated that compared to CAR19-T-cells, a single dose of 2nd generation CAR19-iNKT-cells without in vivo cytokine support, exerts a more effective anti-lymphoma effect in vivo through dual targeting of CD19 by CAR19 and of CD1d, often expressed on B lymphoma cells,[30] by the endogenous iTCR (Fig. 1).[31] Notably, intravenously administered CAR19-iNKT but not CAR19-T-cells swiftly eradicated secondary brain lymphoma.[31] This finding might be related to the higher expression by iNKT-cells of integrins and chemokine receptors required for crossing the blood- and choroidal plexus-brain barriers.[31] Another salient feature was enhancement of the anti-lymphoma effect of CAR19-iNKT-cells by transcriptional upregulation of CD1d expression in lymphoma and CLL B-cells using all-trans retinoic acid (ATRA).[31] Mechanistically, this involved de-repression by ATRA of *CD1D* transcription restrained by the co-operative interaction of RARα with EZH2, a component of the Polycomb repressive complex.[31]

Future perspectives

Taken together the pre-clinical and clinical evidence outlined above lay the foundations for clinical interventional studies that will explore the role of donor iNKT cells in the prevention of aGVHD.

While the pre-clinical potential of CAR-iNKT-cells in other CD1d-expressing malignancies such as multiple myeloma[32] will be doubtless explored, clinical development of CAR-iNKT cells is already under way. Early phase clinical trials aiming to test safety and efficacy of autologous 2nd generation CARGD2-iNKT-cells co-expressing IL-15 for children with neuroblastoma (NCT03294954) and of allogeneic CAR19-iNKT-cells co-expressing IL-15 for B lineage malignancies including CLL and ALL (NCT03774654) have been registered. These and additional studies will define the future role of iNKT-cells as a potentially highly promising and versatile immunotherapy platform in the treatment of B lineage blood cancers and perhaps beyond.

References

1. Salio M, Silk JD, Jones EY, et al. Biology of CD1- and MR1-restricted T cells. *Annu Rev Immunol.* 2014;32:323–366.
2. Bendelac A, Savage PB, Teyton L. The Biology of NKT Cells. *Annu Rev Immunol.* 2007;25:297–336.
3. Godfrey DI, MacDonald HR, Kronenberg M, et al. NKT cells: what's in a name? *NatRevImmunol.* 2004;4:231–237.
4. Dellabona P, Padovan E, Casorati G, et al. An invariant V alpha 24-J alpha Q/V beta 11 T cell receptor is expressed in all individuals by clonally expanded CD4-8- T cells. *J Exp Med.* 1994;180:1171–1176.
5. Gapin L. Development of invariant natural killer T cells. *Curr Opin Immunol.* 2016;39:68–74.
6. Johnston B, Kim CH, Soler D, et al. Differential chemokine responses and homing patterns of murine TCR alpha beta NKT cell subsets. *J Immunol.* 2003;171:2960–2969.
7. Lee PT, Benlagha K, Teyton L, et al. Distinct functional lineages of human V (alpha)24 natural killer T cells. *J Exp Med.* 2002;195:637–641.
8. Gumperz JE, Miyake S, Yamamura T, et al. Functionally distinct subsets of CD1d-restricted natural killer T cells revealed by CD1d tetramer staining. *J Exp Med.* 2002;195:625–636.
9. Chaudhry MS, Karadimitris A. Role and regulation of CD1d in normal and pathological B cells. *J Immunol.* 2014;193:4761–4768.
*10. Chaidos A, Patterson S, Szydlo R, et al. Graft invariant natural killer T-cell dose predicts risk of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. *Blood.* 2012;119:5030–5036.

First clinical observational study to demonstrate association of high graft iNKT-cell dose with protection from clinical aGVHD in - cell replete allo-SCT.

11. Mavers M, Maas-Bauer K, Negrin RS. Invariant natural killer T cells as suppressors of graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. *Front Immunol.* 2017;8:900.
12. Ferrara JL, Levine JE, Reddy P, et al. Graft-versus-host disease. *Lancet.* 2009;373:1550–1561.
13. Ferrara JL. Pathogenesis of acute graft-versus-host disease: cytokines and cellular effectors. *J Hematother Stem Cell Res.* 2000;9:299–306.
14. Schneidawind D, Baker J, Pierini A, et al. Third-party CD4+ invariant natural killer T cells protect from murine GVHD lethality. *Blood.* 2015;125:3491–3500.
*15. Schneidawind D, Pierini A, Alvarez M, et al. CD4+ invariant natural killer T cells protect from murine GVHD lethality through expansion of donor CD4+CD25+FoxP3+ regulatory T cells. *Blood.* 2014;124:3320–3328.

Demonstrates protective effect of donor iNKT cells against experimental aGVHD and their potential interaction with Tregs.

16. Schneidawind D, Pierini A, Negrin RS. Regulatory T cells and natural killer T cells for modulation of GVHD following allogeneic hematopoietic cell transplantation. *Blood.* 2013;122:3116–3121.
17. Leveson-Gower DB, Olson JA, Sega EI, et al. Low doses of natural killer T cells provide protection from acute graft-versus-host disease via an IL-4-dependent mechanism. *Blood.* 2011;117:3220–3229.
18. Yang J, Gao L, Liu Y, et al. Adoptive therapy by transfusing expanded donor murine natural killer T cells can suppress acute graft-versus-host disease in allogeneic bone marrow transplantation. *Transfusion.* 2010;50:407–417.
19. Coman T, Rossignol J, D'Aveni M, et al. Human CD4- invariant NKT lymphocytes regulate graft versus host disease. *Oncoimmunology.* 2018;7:e1470735.
20. Patterson S, Chaidos A, Neville DC, et al. Human invariant NKT cells display alloreactivity instructed by invariant TCR-CD1d interaction and killer Ig receptors. *J Immunol.* 2008;181:3268–3276.
21. Rubio MT, Moreira-Teixeira L, Bachy E, et al. Early posttransplantation donor-derived invariant natural killer T-cell recovery predicts the occurrence of acute graft-versus-host disease and overall survival. *Blood.* 2012;120:2144–2154.

*22. Rubio MT, Bouillie M, Bouazza N, et al. Pre-transplant donor CD4-invariant NKT cell expansion capacity predicts the occurrence of acute graft-versus-host disease. *Leukemia*. 2017;31:903–912.

Similar to Ref 10, demosntrates the role of donor iNKT cells in protection from aGVHD in T-cell-depleted allo-SCT.

23. Exley MA, Friedlander P, Alatrakchi N, et al. Adoptive transfer of invariant NKT cells as immunotherapy for advanced melanoma: a phase I clinical trial. *Clin Cancer Res*. 2017;23:3510–3519.
24. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377:2531–2544.
25. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med*. 2017;377:2545–2554.
26. Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. *Nature*. 2017;543:113–117.
27. Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. *Sci Transl Med*. 2017;9 (374.):

*28. Tian G, Courtney AN, Jena B, et al. CD62L+ NKT cells have prolonged persistence and antitumor activity in vivo. *J Clin Invest*. 2016;126:2341–2355.
*29. Heczey A, Liu D, Tian G, et al. Invariant NKT cells with chimeric antigen receptor provide a novel platform for safe and effective cancer immunotherapy. *Blood*. 2014;124:2824–2833.

Refs 28 and 29 are the first to demonstrate feasibility of engineering CAR-iNKT cells and their anti-tumor activity.

30. Kotsianidis I, Nakou E, Spanoudakis E, et al. The diagnostic value of CD1d expression in a large cohort of patients with B-cell chronic lymphoproliferative disorders. *Am J Clin Pathol*. 2011; 136:400–408.
31. Rotolo A, Caputo VS, Holubova M, et al. Enhanced anti-lymphoma activity of CAR19-iNKT cells underpinned by dual CD19 and CD1d targeting. *Cancer Cell*. 2018;34:596–610. e11.
32. Spanoudakis E, Hu M, Naresh K, et al. Regulation of multiple myeloma survival and progression by CD1d. *Blood*. 2009;113: 2498–2507.