INVITED SPEAKER PRESENTATION

Open Access

S77. Proffered paper: In vitro induced response patterns of antileukemic T-cells – characterization by combination of functional assays, spectratyping and next generation sequencing

S Reuther^{1*}, P Krell², FR Schuster², C Grabrucker¹, A Liepert¹, T Kroell¹, HJ Kolb¹, A Borkhardt², R Buhmann¹, H Schmetzer¹

From 1st Immunotherapy of Cancer Conference (ITOC1)
Munich, Germany. 12-14 March 2014

Background

T-cell receptor (TCR) diversity is characterised by somatic alterations in the complementary determining region 3 (CDR3) of the human TCR β -chain. Complemented with the TCR alpha-chain, TCR diversity can hypothetically result in up to 10^{18} different TCR molecules.

Myeloid leukaemic cells can be induced to differentiate into leukaemia-derived dendritic cells (DC_{leu}) regaining the stimulatory capacity of professional DCs while presenting the whole leukaemic antigen repertoire.

Our aim was to identify TCR $V\beta$ -chain-rearrangements in T-cells stimulated with leukaemic blasts and DC_{leu} in 3 patients with AML and furthermore to detect, amplify or monitor T-cell clones with defined $V\beta$ -profiles in correlation with antileukaemic function, in vitro and in vivo.

Material and methods

HLA matched or HLA haplo-identical (allogeneic) donor- or autologous T-cells were repeatedly stimulated, either with leukaemic blasts or the corresponding DC_{leu} from 3 different AML-patients. Cytotoxicity assay was carried out for measuring the lytic activity of effector T-cells, spectratyping was performed to identify the restriction of the TCR $V\beta$ -repertoire in unstimulated and stimulated T-cells and Sanger sequencing to analyse the β -chain sequence information including the CDR3 regions. Additionally next generation sequencing (NGS) was established to

analyse the accurate TCR sequence information of thousands of TCR β -chains with high coverage.

Results

No significant differences in T-cell proliferation were observed. The T-cell mediated cytolytic response patterns showed blast lysis (n=1) and blast proliferation (n=2).

Spectratyping revealed a remarkable TCR $V\beta$ -restriction of the CD4⁺- or CD8⁺-TCR repertoire of blast- or DC/DC_{leu}-stimulated T-cells, independently of blast or DC/DC_{leu} used as stimulators. Although in absolute terms, DC/DC_{leu} stimulation induced the highest grade of restriction in the CD8⁺ T-cell subset, the CD4⁺ T-cells seemed to be relatively more affected.

In vitro stimulation with DC/DC_{leu} resulted in an identical TCR (β -chain restriction pattern) as identified in vivo in a patient sample 3 months after allogeneic stem cell transplantation (SCT).

Conclusion

A combined strategy using spectratyping and NGS with functional tests may provide useful information about the specificity and efficacy of the intra-individual variable induced T-cell response.

Spectratyping allows the identification of a restricted $V\beta$ -repertoire by Gaussian-like distribution, NGS allows sequencing of TCR repertoires with high coverage, novel software allows the analysis of the exact length and sequence composition (the combination of the $V\beta$ - and J β -genes) of the β -chains, especially of the CDR3, and the exclusion of non-functional transcripts.

¹University of Munich, Med. III Hematopoietic Transplantations, Munich, Germany

Full list of author information is available at the end of the article

The identification of defined V β -T-cell clones may lead to selection procedures generating Graft-versus-Leukaemia reaction- but not Graft-versus-Host disease-mediating T-cells for adoptive immunotherapy after SCT.

Authors' details

¹University of Munich, Med. III Hematopoietic Transplantations, Munich, Germany. ²University of Duesseldorf, Department of Pediatric Oncology Hematology and Immunology, Duesseldorf, Germany.

Published: 12 March 2014

doi:10.1186/2051-1426-2-S2-I15

Cite this article as: Reuther *et al.*: S77. Proffered paper: In vitro induced response patterns of antileukemic T-cells – characterization by combination of functional assays, spectratyping and next generation sequencing. *Journal for ImmunoTherapy of Cancer* 2014 **2**(Suppl 2):115.

**Submit your next manuscript to BioMed Central
and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit