PF204

TARP AS IMMUNOTHERAPEUTIC TARGET IN AML EXPRESSED IN THE LSC COMPARTMENT

B. DEPRETER[1,2], K. E. WEENING[2,3], K. VANDEPOELE[2,4] M. ESSAND[5], B. DE MOERLOOSE[1,2,6], M. THEMELI[7], J. CLOOS[7], D. HANEKAMP[7], I. MOORS[8], I. D'HONT[6], B. DENYS[2,4], A. UYTTEBROECK[9], A. VAN DAMME[10], L. DEDEKEN[11], S. SNAUWAERT[12], G. GOETGELUK[3], S. DE MUNTER[2,3], T. KERRE[2,8], B. VANDEKERCKHOVE[2,3], T. LAMMENS[1,2*] and J. PHILIPPE[2,3,4*]

[1]Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium;[2]Cancer Research Institute Ghent, Ghent University, Ghent, Belgium;[3]Department of Diagnostic Sciences, Ghent University, Ghent, Belgium;[4]Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium;[5]Science for Life Laboratory, Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden;[6]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium;[7]Department of Hematology, VU University Medical Center, Amsterdam, the Netherlands;[8]Department of Hematology, Ghent University Hospital, Ghent, Belgium;[9]Department of Pediatrics, University Hospital Gasthuisberg, Louvain, Belgium;[10]Department of Pediatric Hematology Oncology, University Hospital Saint-Luc, Brussels, Belgium;[11]Department of Pediatric Hematology Oncology, Queen Fabiola Children's University Hospital, Brussels, Belgium;[12]Department of Hematology, AZ Sint-Jan Hospital Bruges, Bruges, Belgium. *shared last authors

BACKGROUND

- Despite frequent initial remission, adult and pediatric AML (pedAML) patients exhibit a high relapse risk, probably due to therapy-resistant leukemic stem cells (LSC).
- The prognosis of FLT3-ITD mutated patients is extremely poor.
- Current chemotherapeutic regimens perform inadequate towards LSC eradication.
- The TCRγ chain alternate reading frame protein (TARP) was identified as an immunotherapeutic target in prostate and breast adenocarcinoma.

OBJECTIVES

Identify LSC aberrations and develop LSC-targeted strategies while assuring salvage of normal hematopoietic stem cells (HSC).

MATERIALS & METHODS

The GSE 17054 micro-array dataset was used for transcriptome analysis of CD34+CD38- populations sorted from *de novo* adult AML (LSC=9) and healthy adults (HSC=4). We next evaluated transcript expression in CD34+CD38- and CD34+CD38+ sorted populations from *de novo* pediatric AML (pedAML) and healthy controls. First, an 8x60K human gene expression micro-array was performed in 4 pedAML (2 FLT3 WT, 2 FLT3-ITD) and 3 cord blood (CB) samples. Second, qPCR was performed in 13 pedAML, 17 adult AML, and 15 healthy controls (7 CB, 6 normal pediatric bone marrow (NBM) and 2 mobilized blood stem cells (mPBSC), as well as cell lines of various origin (AML=9, B-ALL=5, CML=1, B-NHL=2). qPCR data analysis was performed according to the state-of-the-art and calibrated normalised relative quantities (CNRQ) were calculated.

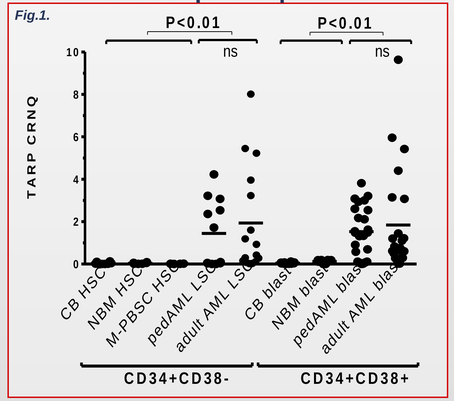
Protein expression was evaluated in AML cell lines, including transgenic TARP-overexpressing and shRNA-induced TARP-knockdown cell lines, and in pedAML leukemic cells by Western blotting and confocal microscopy.

TARP-redirected T-cell receptor (TCR)-engineered cytotoxic T-cells (CTLs) were generated by lenti- (LV) and retroviral (RV) transduction. Targetability of native and transgenic AML cell lines and primary patient leukemic cells was examined *in vitro* by flow cytometric (FCM) cytotoxicity assays.

RESULTS

1. TARP transcript is expressed in adult and pediatric AML blasts and LSC

Fig.1.



In the GSE 17054 dataset, TARP was shown to be most differentially expressed between adult LSC and HSC (log2-FC 6.92, P<0.01). qPCR analysis of CD34+CD38+ and CD34+CD38- fractions confirmed a significant higher TARP expression in pediatric and adult leukemic blasts and LSCs compared to their normal counterparts (***Fig.1***). In addition, TARP transcript levels were significantly increased in AML cell lines derived from pediatric cases (MV4;11, THP-1), LSC-enriched cell lines (Kg-1a, HNT-34) (P<0.001), and HL-60, whereas negative in B-ALL/NHL and CML.

2. TARP expression is correlated with a worse prognosis in pedAML

Fig.2A.

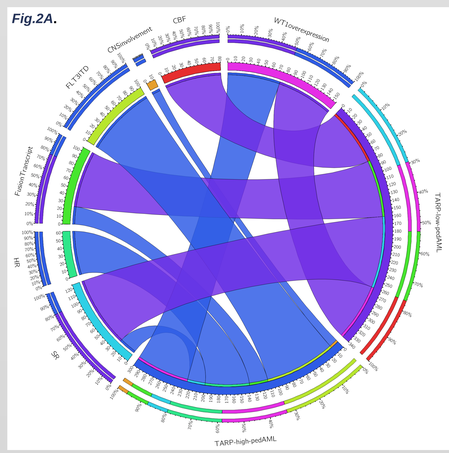
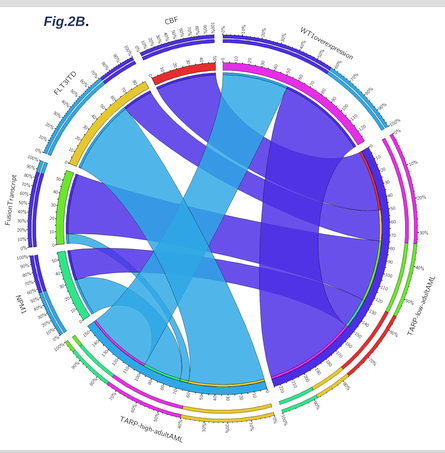


Fig.2B.



The number of patients harboring FLT3-ITD (P<0.001) and HR profiles (P<0.05) were significantly higher in TARP-high pedAML, whereas TARP-low pedAML patients included significantly more CBF-leukemia (P<0.01) and SR profiles (P<0.05) (***Fig.2A***). In adult AML, high TARP expression was not restricted to FLT3-ITD (***Fig.2B***).

3. TARP protein expression is correlated to transcript levels

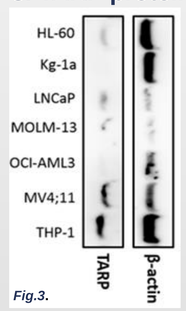


Fig.3.

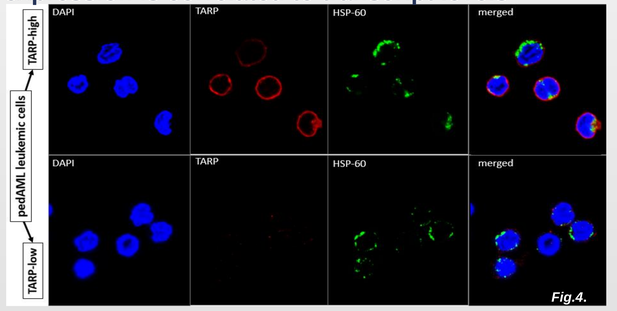
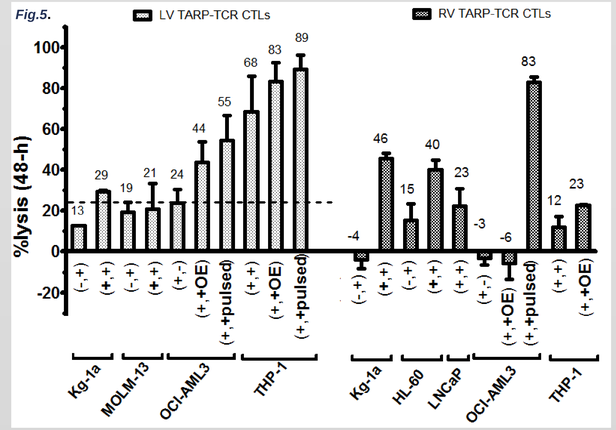


Fig.4.

TARP protein expression was confirmed by Western Blotting in cell lines in agreement with transcript levels (***Fig.3***). Confocal microscopy visualised TARP (red) together with DAPI (blue) and HSP-60 (green, mitochondrial marker). TARP stained in leukemic cells sorted from a TARP-high pedAML patient, whilst remaining negative in TARP-low leukemic cells (***Fig.4***).

4. TARP and HLA-A*0201 co-expressing cell are killed *in vitro* by TARP-TCR CTL

Fig.5.



Legend to Fig.5.
*Mean lysis (%) is shown and whiskers represent ±SEM. The dashed line indicates the highest level of non-TARP mediated background killing observed for LV TARP-TCR CTLs, whereas for RV transduced CTLs, mock CTLs were constructed. Positive (+) or negative (-) expression for HLA-A*0201 and TARP is shown between brackets in this respective order. Bold symbols indicate the expression differing from wild-type (WT), either by retroviral transduction or pulsing.*

The lytic response of TARP-TCR CTLs towards WT, transgenic and pulsed AML cell lines (***Fig.5***) showed that HLA-A*0201 transgenic AML cell lines were more efficiently lysed compared to their HLA-A*0201-negative counterparts. Also, a higher lysis was observed for TARP-transgenic and -pulsed cell lines compared to their WT. Lysis of leukemic cells from adult AML (n=5) borderline correlated to TARP transcript levels (Spearman's ρ=0.82, P=0.089).

CONCLUSIONS

- **TARP is an AML-specific target expressed in the LSCs and blasts of pediatric and adult AML, while absent in their normal counterparts.**
- **TARP transcript expression is associated with FLT3-ITD mutations in pedAML.**
- **TARP-TCR transgenic CTLs hold great promise as a novel immunotherapeutic strategy in AML.**