Anti-CSPG4 CAR.CIK lymphocytes are effective against advanced sarcomas in 3D spheroid and xenograft models

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Abstract 885: Anti-CSPG4 CAR.CIK lymphocytes are effective against advanced sarcomas in 3D spheroid and xenograft models FREE

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Abstract

Purpose of this study is to explore, *in vivo* and within tridimensional (3D) models, a novel CAR-based adoptive immunotherapy against incurable soft tissue sarcomas (STS). The strategy focuses on Cytokine-Induced Killer (CIK) lymphocytes redirected against the Chondroitin Sulfate Proteoglycan 4 (CSPG4) target, associated with tumorigenesis, tumor aggressiveness and negative clinical outcome. CIK are ex vivo expanded T-NK lymphocytes endowed with intrinsic HLA-independent antitumor activity.

Experimental procedures. The experimental platform is based on patient-derived CAR.CIK and multiple histotypes of STS. CAR.CIK were generated by transduction of PBMC with a retroviral vector encoding for a 2nd generation anti-CSPG4 CAR with 4-1BB costimulation. 3D in vitro essays were based on STS spheroids generated in ultralow attachment conditions. In vivo experiments included 3 different STS xenograft models (fibrosarcoma, leiomyosarcoma, UPS).

Results. CAR.CIK were efficiently generated by STS patients (n=5). Mean expression of anti-CSPG4 was 44%±6.2, rates of *ex vivo* expansion (29 fold, range 27-348) and phenotype (CD8: 65%±4; CD56: 38%±6, NKG2D: 66%±7) were comparable with unmodified controls (NTD.CIK.). The CSPG4 target resulted expressed in 16/17 STS (leiomyosarcoma n=2, fibrosarcoma n=1, UPS n=6, GIST n=5, liposarcoma n=3), with variable membrane density per cell (300±47). CAR.CIK efficiently killed all STS *in vitro* regardless of their histotype. Mean STS-specific killing by CAR.CIK was significantly higher compared with NTD.CIK (E:T 1:1: 71% vs 34%, p <0.0001). Within 2 different STS 3D-spheroid models, CAR.CIK showed higher penetration ability through Matrigel matrix (n=5 p≤0.05), tumor recruitment, infiltration (n=8, p≤0.01) and killing (n=3, p<0.0001) compared with NTD.CIK. Anti-STS activity by CAR.CIK appeared proportionally dependent on CSPG4 density in STS targets. We set 3 different STS xenografts (fibrosarcoma,

leiomyosarcoma, UPS) in immunocompromised mice, differing for CSPG4 expression and density levels per cell. Treatment with CAR.CIK (autologous in 2/3 cases) determined a significant delay of tumor growth ($p<0.0001$) compared with controls, demonstrating intense STS infiltration after treatment. A persistent antitumor response was observed up to 2 weeks after end of treatment. Also in vivo, we confirmed a positive correlation between the observed anti-STS activity of CAR.CIK and target CSPG4 expression/density on tumor cells.

Conclusions. We report the intense activity of anti-CSPG4 CAR.CIK against multiple histotypes of currently incurable STS. CIK lymphocytes, considering their intrinsic antitumor activity, may be a favorable platform for the translation of CAR-based strategies against solid tumors. Our findings support anti-CSPG4 CAR.CIK as a promising therapeutic strategy warranting clinical exploration in the challenging field of advanced STS.

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