Generation of T-cell lines with broadened antigenic specificities to improve adoptive immunotherapy protocols for the treatment of Nasopharyngeal Carcinoma.


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AIM: To develop optimized, GMP-upgradable protocols for adoptive immunotherapy of NPC based on the generation of T-cell lines enriched in BARF1-specific effectors.

METHODS: Lymphoblastoid cell lines (LCLs) were treated with different EBV lytic cycle inducers of doses able to induce abortive or limited EBV replication while preserving cell viability. Expression of BARF1, LMP1, ZEBRA and EA mRNA was quantified by qRT-PCR. EBV-specific donor- and patient-derived CTLs were generated with LCLs treated with the different drugs from PBMCs. Standard cytotoxicity assays were used to assess the specificity of CTLs. Content in Granulysin B granules was assessed by multiplex imaging flow cytometry.

RESULTS:

1. DX induces a specific up-regulation of BARF1 mRNA and an abortive lytic cycle activation (EA, ZEBRA) in donor-derived LCLs

Treatment with low doses of dexamethasone (DX) proved to be the most suitable and simple protocol to enhance BARF1 expression (3.3 fold increase) without down-regulating other viral antigens that also get targeted by EBV-specific CTLs. By contrast, TPA/Na-butyrate (TPA+Nab) or cisplatin (CSP) was less effective in up-regulating BARF1 or induced higher levels of cell apoptosis, assessed by PARP cleavage in Western blot.

2. Our protocol provides effectors with a memory profile similar to standard CTLs

3. Generation of cellular model for cytotoxicity assay: c666.1 cells stably expressing HLA-A*0201

4. DX-CTLs selectively recognize and kill EBV+ cell lines and BARF1-loaded targets with more efficacy than TPA+Nab- and cPI-CTLs.

CTLs induced with DX-treated LCLs (DX-CTLs) showed high levels of specific cytotoxicity against NPC cells endogenously expressing BARF1 (c666.1.A2, >90% of specific lysis) or T2A2 cells loaded with BARF1 or LMP1. LHA-A2 peptides (30% specific lysis). CTLs generated with LCLs either untreated or exposed to TPA+Nab of CSP induced only low levels of BARF1 or LMP1 specific cytotoxicity. Notably, the extent of specific lysis induced by DX-CTLs was higher against the BARF1- peptides.

5. DX-CTLs display an increased frequency of effector-target doublets formation and a higher content of granulysin B granules

Cytotoxicity assay simulation: peptide-loaded-LCLs co-cultured with CTLs for 2 Hrs

Multiplex imaging flow cytometry

6. DX enables LCLs to more efficiently present antigens, probably through HLA-A*0201 up-regulation and activation of immunogenic cell death associated molecules (DAMPS)

7. DX-LCLs derived from UNMC patients show activation of DAMPS and an increased expression of BARF1 and LMP1 mRNA. DX-LCLs display high specificity and killing ability

CONCLUSIONS: These findings provide the rationale for a rapid up-grading at the GMP level of the use of DX-treated LCLs for the generation of CTL lines enriched in BARF1 specificities for adoptive immunotherapy of resistant or relapsing NPC.