Challenging GD2-Specific CAR T Cells Against Patient-Derived Glioblastoma Cells

Malvina Prapa[1], Chiara Chiavelli[1], Chiara Sautto[1] , Carlotta Spano[2], Giulia Grisendi[2], Olivia Candini[2], Elisabetta Manuela Foppiani[2], Giulia Golinelli[1]
Sara Caldrer[3], Marco Bestagno[4], Monica Cellini[5], Dario Campana[6], Paolo Paolucci[5], Elena Zunarelli[7]
Alberto Feletti[8], Giacomo Pavesi[8] & Massimo Dominici[1]

[1]Division of Oncology, University-Hospital of Modena and Reggio Emilia, Modena, Italy; [2]Rigenerand Srl, Medolla, Modena, Italy; [3]Department of Pathology and Diagnostics, University of Verona, Verona, Italy; [4]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy; [5]Pediatric Oncology Unit, University-Hospital of Modena and Reggio Emilia, Modena, Italy; [6]Department of Pediatrics, National University of Singapore, Singapore; [7]Department of Pathology, University Hospital, Modena, Italy;
[8] Department of Neurosciences, Unit of Neurosurgery, NOCSAE Modena Hospital, Baggiovara, Italy

INTRODUCTION

Disialoganglioside GD2 is a membrane antigen overexpressed in several pediatric and adult cancer types such as brain tumors like glioblastoma, neuroectoderm-derived tumors and sarcomas [1]. GD2 has been actively targeted for cancer immunotherapy approaches [2][3].

Among immunotherapies**, CAR-T cells** are becoming promising in hematology while they are still struggling for solid tumors. CARs are recombinant receptors that redirect the specificity and functions of T lymphocytes and of other immune cells to antigens expressed on the surface of tumor cells in an MHC-independent manner. Upon antigen recognition, CAR T cells execute effector functions, including the production of antitumor cytokines and the killing of target cells, thus acting as “living drugs” that potentially would exert both immediate and long-term therapeutic effects [4].

Glioblastoma (GBM) is the most malignant and frequent primary glial brain tumor. Although gradual improvements in survival rates and quality of life for GBM patients have been noticed, the medical need is still widely unmet. The location and the infiltrative capability of GBM make complete surgical resection difficult to be achieved; moreover, chemotherapy and irradiation are not yet curative, with patient majority experiencing tumor progression or recurrence with an average survival of approximately 12 months [5]. **In this perspective, immunotherapy approaches by wild type and gene modified T cells may provide a new strategy to target GBM.**

Starting to these assumptions, we have challenged the anti-GD2-BB-ζ CAR (GD2 CAR) activity targeting patient GD2-positive glioblastoma cells. The receptor was expressed in human T cells by retroviral transduction and anti-tumor activities were assessed *in vitro* by 2D and 3D cytotoxic assays.

METHODS

Patients and samples. The study was approved by the Ethical and Institutional Review Board at the University Hospital of Modena and was carried out in accordance with the relevant guidelines and regulations. Tumor samples and peripheral blood were obtained after signed informed consent from patients who underwent surgery at the Neurosurgery Unit (NOCSAE, Baggiovara, University Hospital of Modena).

Isolation and maintenance of GBM cells in culture. High grade diagnosed glioma samples were dissociated into single cells with Human Tumor Dissociation Kit (MACS, Myltenyi Biotec) using gentleMACS Octo Dissociator with heaters protocol’s (Myltenyi Biotec) and plated in stem cell media, DMEM-F12 supplemented with B27 1x (Gibco), EGF (20 ng/ml, PeproTech), bFGF (20 ng/ml, PeproTech), 1% glutamine and 1% penicillin-streptomycin. Once cells start forming spheroids, medium is replaced every 2-3 days by letting them settle to the bottom by gravity at 37°C. The cell passage was performed approximately every 5 days in order to prevent spheroids from growing too large.

Effector cells. Peripheral blood mononuclear cells (PBMC) were separated by density gradient (Lymphoprep; Fresenius, Axis-Shield, Oslo, Norway) and then plated in RPMI 1640 with 1% FBS, 1% glutamine and 1% penicillin-streptomycin. Non-adherent cells were collected and pre-stimulated for 48 hours in RPMI 1640 supplemented with 10% heat-inactivated defined FBS, 500 UI/mL rhInterleukin-2 (rhIL-2, Proleukin, Novartis Farma S.p.a) and 7 µg/mL Phytohemagglutinin (PHA-M, Sigma-Aldrich) at the concentration of 1 $\times 10^6$ cells/mL.. For CAR-modified T cell generation, manufacturing and immunophenotype refer to Prapa et al. [6].

Cytotoxicity assays. GD2 CAR T or GFP only T cells were used as effectors and patient GD2-positive primary glioblastoma cells as targets (previously dsRED transduced with retroviral vectors). GBM cells incubated with medium alone or 1% Triton X-100 were used as controls, representing 100% and 0% cell viability, respectively. Average viability was calculated as 100 x (experimental fluorescence - 0% viability fluorescence)/(100% viability fluorescence - 0% viability fluorescence) using GloMax Discover Multimode Microplate Reader (Promega, Madison, WI, USA).

Spheroid assay. 20.000 cells/well seeding density was used in order to reach sphere formation after 24h, by then effector cells in E:T ratio of 2:1 were co-cultered with tumor spheres and co-cultures were monitored over 5 days.

3D co-culture model. A novel 3D bioreactor, VITVO® (Rigenerand Srl, Medolla, Modena, Italy) was used to assess co-cultures of target cells with effector T cells at an E:T ratio of 5:1 over 72h and 5 days. LIVE (CalceinAM) /DEAD (EtBr) Kit (Life Technologies Co) was used to assess the Viability/Cytotoxicity rate in imaging. The apoptotic rate was assessed with RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay (Promega).

CONCLUSION

In this study, we propose the disialoganglioside **GD2 antigen as a valuable target for CAR T cell therapy to treat GBM**. The preliminary data provided *in vitro* redirected activity of GD2 CAR T cells towards patient GD2-positive glioblastoma cells, further validating our approach similarly to what we reported in a previous study dealing with GD2-positive neuroblastoma cells in vitro and in a xenograft model [6].

Transduced T cells expressed high levels of anti-GD2 CAR and exerted a robust and specific anti-tumor activity over different time points in 2D and 3D co-culture assays. A novel 3D bioreactor was employed over 5 days of co-cultures to assess cytotoxicity in a GBM tissue-like structure showing a robust apoptotic effect and major presence of infiltrative effector elements in respect to the control condition.

Collectively, these results warrant a future challenging of the GD2-CAR platform to treat patient-derived orthotopic glioblastoma models and, in prospective, affected patients.

RESULTS

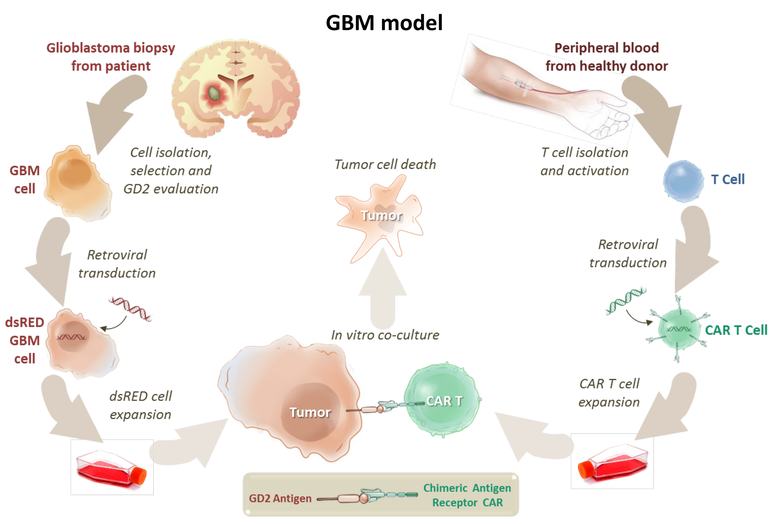


Fig. 1. 48h co-culture assay, E:T= 2:1, fluorescence reading

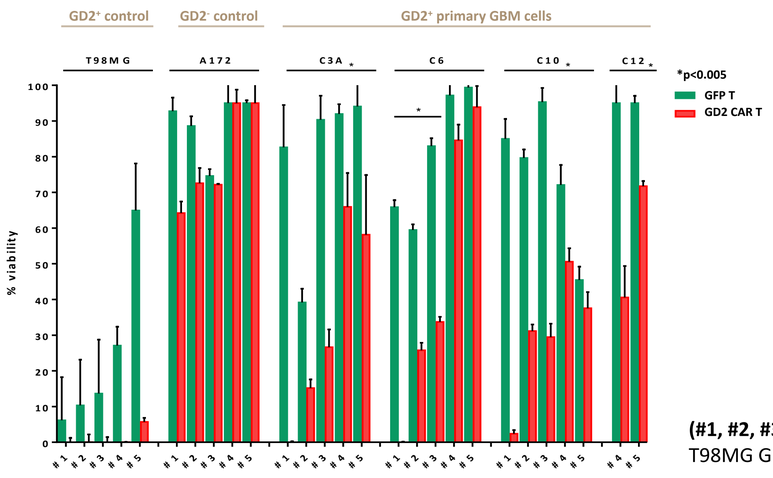
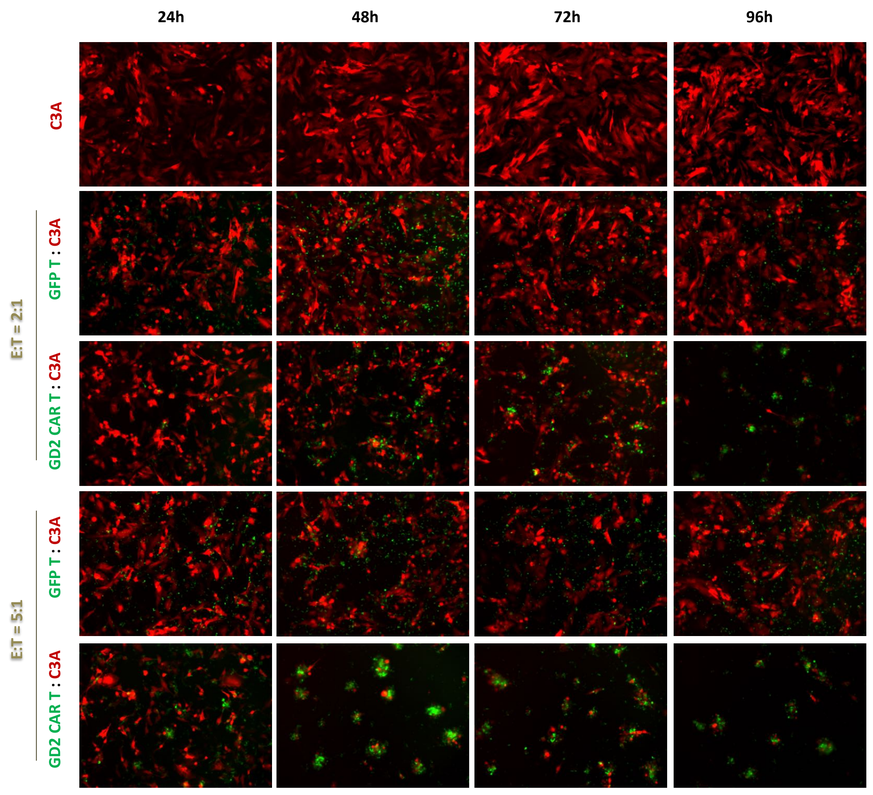


Fig. 2. Co-cultures with patient C3A cells, representative photomicrographs (10x)



(#1, #2, #3, #4, #5) T cell donors, **(C3A, C6, C10, C12)** patient **GD2-positive** GBM cells
T98MG GD2$^+$ control, A172 GD2$^-$ control

Fig. 3. Spheroid co-cultures with patient C3A cells, E:T= 2:1 (4x)

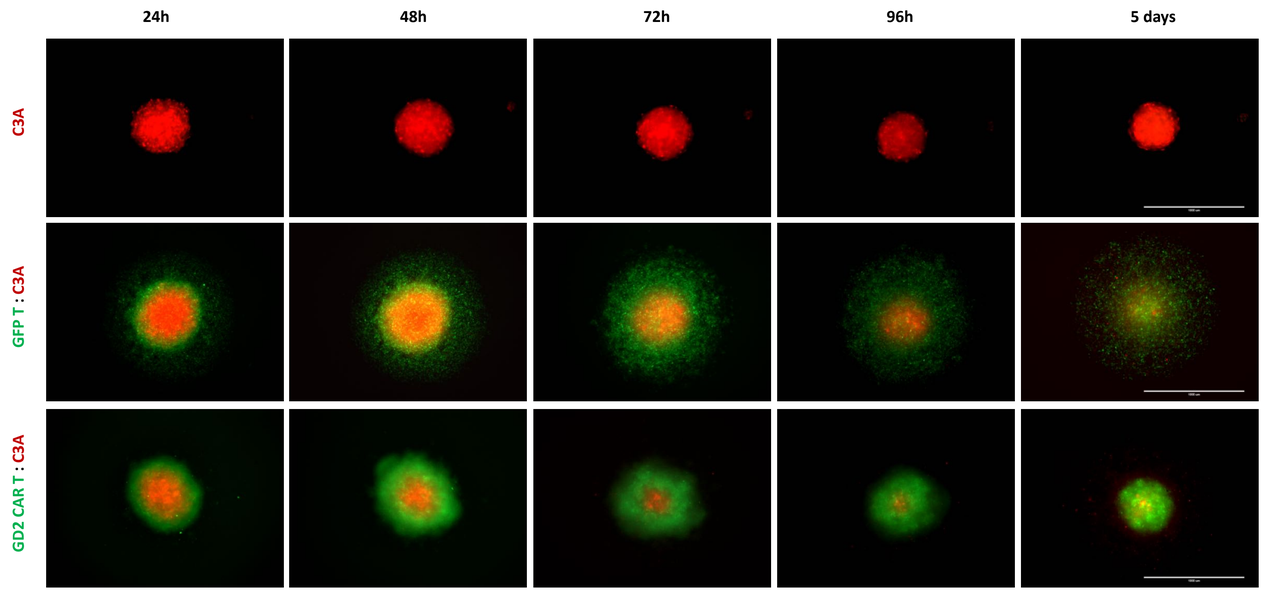


Fig. 4.a 3D co-culture model with patient C3A cells, E:T= 5:1, LIVE (CalceinAM) & DEAD (EtBr) assay (4x)

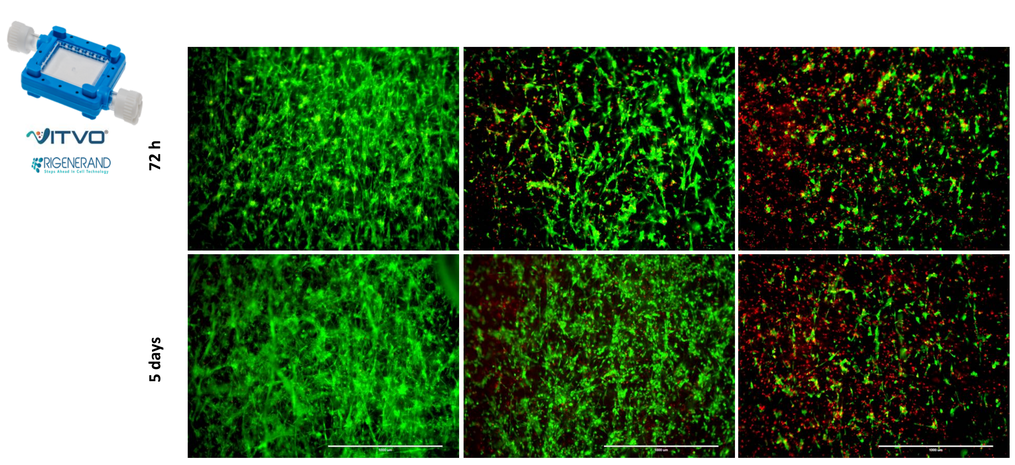
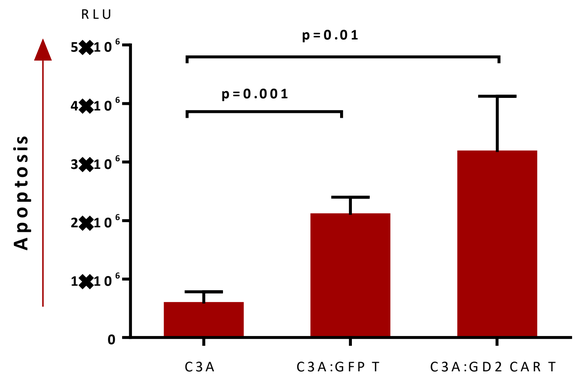


Fig. 4.b Apoptosis rate after 24h co-culture



REFERENCES

[1] Ahmed M et al. FEBS Lett, 2014
[2] Suzuki M et al. Expert Opin Ther Targets, 2015
[3] Dobrenkov K et al. Semin Oncol, 2014
[4] Eshhar Z. Hum Gen Ther, 2014
[5] Hanif F. Asian Pac J Cancer Prev APJCP, 2017
[6] Prapa M et al. Oncotarget, 2015

malvina.prapa@unimore.it

ACKNOWLEDGMENTS

AIRC
ASEOP