Development and validation of a fully GMP-compliant production process of autologous, tumor-lysate pulsed dendritic cells

Eyrich M[1], Schreiber SC[1], Rachor J[1], Pauwels F[3], Wölfl M[1], Schlegel PG[1], Schrauth B[1], Lutz MB[2], van Gool S[3]

[1]Stem cell laboratory, Children's Hospital Würzburg, [2]Institute of Virology and Immunobiology, University of Würzburg, Germany

[3] Experimental neurooncology, University Children's Hospital Leuven, Belgium on behalf of the HGG-Immuno network

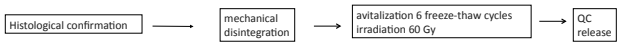
Poster #135

Abstract

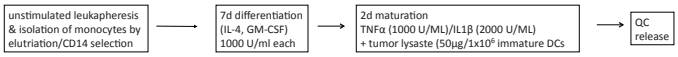
Background One of the major challenges of DC vaccination is the recruitment of further cell therapy facilities and the establishment of validated and harmonized DC-production protocols in order to conduct larger, randomized clinical trials. Here, we report about the transfer and validation of a former successfully used open DC-generation method into a closed-system, GMP-compatible protocol. **Methods** A previously published DC-generation protocol using ficollized PMNC and plate adherence was stepwise translated into a closed system using large-scale monocyte isolation techniques and culture in teflon bags with GMP-compatible reagents. All production steps (lysate generation, monocyte selection, DC culture and cryopreservation) were validated and finally approved by competent authorities. **Results** Tumor lysate was characterized by histology, mechanically homogenized and avitalized. Protein measurement showed a median of 53µg protein per mg tumor tissue (n=5). Avitality was proven in an ATP-release assay with a sensitivity of down to 10 cells/preparation. Patient monocytes were isolated by elutriation or CD14-selection. Both methods yielded similar results and were considered equal. DCs were subsequently generated in teflon bags for a optimum of 7 days in CellGro® medium supplemented with IL-4 and GM-CSF and then finally matured in TNFa and IL-1ß under the presence of 50 µg tumor-lysate/1x10^6 DCs. This protocol resulted in robust and reproducible upregulation of DC maturation markers like CD80, CD83, CD86 and HLA-DR. Functionality of these DCs was shown by directed migration towards CCL19/21, positive T-cell stimulatory capacity and the ability to prime antigen-specific T cells from naïve CD8$^+$ T cells. Finally, mature DCs were aliquoted and cryopreserved in Cryostor5®. Vitality and functionality of thawed DCs were extensively validated and showed no significant loss of function. **Conclusion** Our simple, robust, validated and approved protocol for DC-generation forms the basis for a harmonized procedure to produce cancer vaccines, which paves to way for larger multicenter clinical trials.

Methods

a) production of tumor lysate



b) production of dendritic cell vaccines

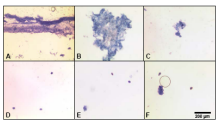


Results

I. Tumor lysate preparation (high-grade gliomas)

trypan-blue staining raw material

trypan-blue staining after Ficoll



	tumor 1	tumor 2	tumor 3	tumor 4	tumor 5
weight [mg]	405.6	512.6	229.8	1370	1460
protein [mg]	27.7	21.9	16.3	68.1	93.5
µg protein/ mg tumor	72	48.8	23.4	49.7	64

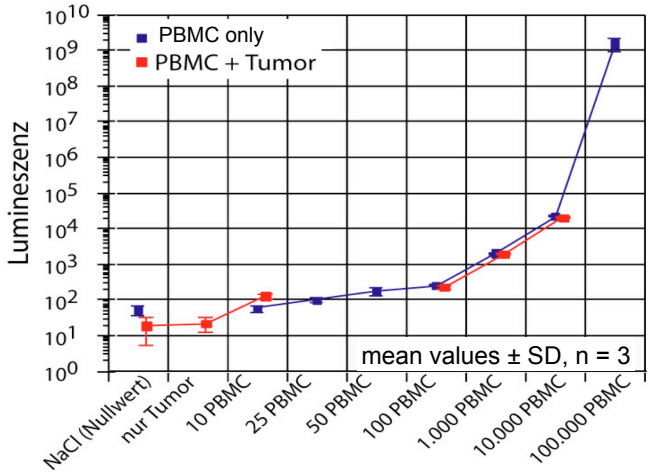
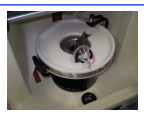


Fig. 1: Detection of ATP as a means to detect residual vital cells. Cells were lysed and assayed by bioluminescence for the release of ATP. Fresh PBMC (blue line) were used to generate a titration curve (positive control). Detection limit of this assay is in the range of prox. 25 cells. Tumor lysate was analyzed purely, or spiked with 10^1, 10^2, 10^3, and 10^4 PBMCs (red line).

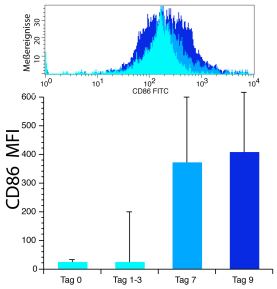
II. Elutriation

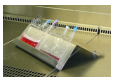


n= 10 validation runs
purity: monocytes 81.3±7.9%,
CD3$^+$ 1.5±0.7, CD19$^+$ 3.8±3.6%

n=1 patient (30 kg), purity 72%, recovery CD14$^+$ 40%

III. DC generation





cytokines
media
bags
from:

CellGenix

Fig. 2: DCs were differentiated for 7 days in IL-4 and GM-CSF and matured for 2 days in the presence of TNFα, IL-1ß and tumor lysate. Increase of CD86 and HLA-DR MFI as surrogate markers for maturation.

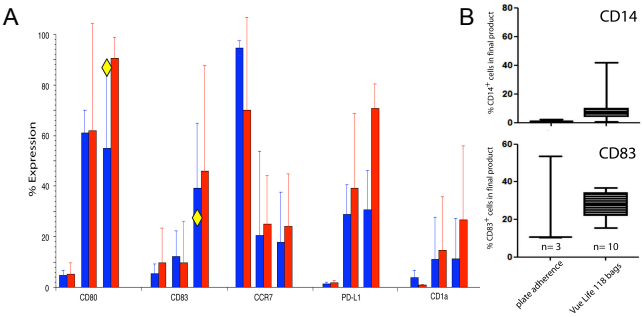


Fig. 3: A) additional phenotypic characterization of mature DCs. Data are shown on days 1, 7, and 9 for elutriated (blue) or CD14-isolated monocytes (red). Yellow rhombs represent data from a GBM patient. B) comparative data on CD14 and CD83 expression using a pre-GMP plate adherence method (left) and the closed DC culture in Vue Life 118 bags (right columns).

IV. Functional characterization of matured DCs

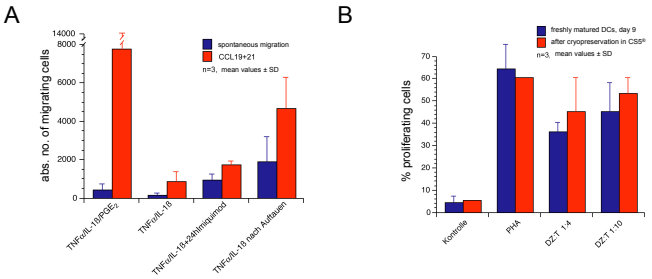


Fig. 4: matured DCs were either used freshly or after cryopreservation in CryoStor5®
A) migratory capacity towards CCL19+21 in a transwell assay, PGE_2-matured DCs served as a positive control. B) T-cell stimulatory capacity pre/post cryopreservation in an alloMLR

Conclusions

- → **fully validated GMP-compatible DC generation protocol**
- → **already used in n=168 high-grade glioma patients (clinical trials of the HGG-Immuno group)**
- → **approved by German (Paul-Ehrlich Institute) and Belgian supreme agencies for cellular therapeutics and vaccines**
- → **standard protocol, platform for further development**