Immunomodulation as one of the key hallmarks of PUVA therapy: results from a mouse lymphoma skin model

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Background

Cutaneous T-cell lymphoma (CTCL) represents heterogeneous group of neoplastic disorders characterized by chronic inflammation and primary accumulation of malignant T cells in the skin [1]. Lesional skin of CTCL patients is colonized by T-cells with aberrant phenotype and non-malignant immune cells, both possibly driven by soluble inflammatory mediators.

PUVA (Psoralen + UVA) is one of the gold standard of treatment for early stages of CTCL [2]. The combination of psoralen and UVA irradiation is frequently used to treat these patients with a success rate of more than 70% of complete clinical response.

DNA damage in malignant cells triggered by PUVA is thought to be the main mechanism of action of this therapy, however, the significance of PUVA-induced DNA and membrane damage-related immunomodulation and its potential contribution to clearance of CTCL lesions remains to be elucidated.

Hypothesis & Aims

Immunomodulation is one of the key hallmark of PUVA mediated therapeutic effects in cutaneous T cell lymphoma

Aims: Mapping the pathways of PUVA mediated immunomodulation in CTCL

⇒ Analyzing T cell population

⇒ Analyzing transcription factors and mapping their role in PUVA mediated immunomodulation

Establishment of murine lymphoma skin model

To get a better understanding of how PUVA works we established a murine model of CTCL where we injected EL-4 cells subcutaneously on the back of a C57BL/6 mice and treated them with PUVA therapy.
Animals were sacrificed at various time points to collect tumor tissue and analyze lymphocytic distribution.

Fig 1:

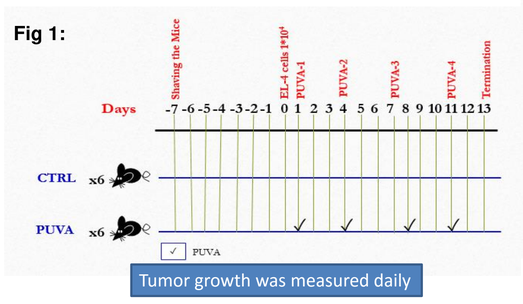


Figure 1: After EL-4 cell injection PUVA were done on Day 1, 4, 8 and 11 (2x/Week), and animal were sacrificed after 2weeks and tumor tissue, blood, spleen and lymph nodes were collected for further analysis.

Fig 2:

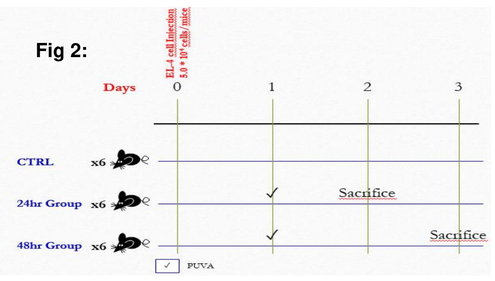


Figure 2: PUVA were done after 24hr of EL-4 cell injection and animals were sacrificed 24 and 48 hr post PUVA exposure. Tumor tissue, blood, spleen and lymph nodes were collected for further analysis.

Materials & Methods

PUVA dose: The mice were painted on their back with either 200 µl of 8-methoxypsoralen (8-MOP) in ethanol (0.1 mg/ml) or 200 µl of vehicle (95% ethanol). UVA irradiation was performed only on psoralen-painted mice after 15 min using a Waldmann Medizintechnik UV 236 A therapy lamp (Villingen-Schwenningen, Germany), filtered for the emission at 315–400 nm at a distance around 12 cm from the dorsal skin of the mouse. The UVA dose used was 1.5 J/cm^2 [3].

To study the systemic immunosuppressive effect of PUVA two separate experiments were performed. In one experiment (Figure 2) mice were sacrificed at 24 and 48 hr post one exposure of PUVA treatment and in other experiment (Figure 1) the PUVA treatment was performed two times a week for two weeks.

⇒ Flow Cytometry were used to analyze lymphocytic distribution and immunomodulatory transcription factors in blood spleen and lymph nodes.

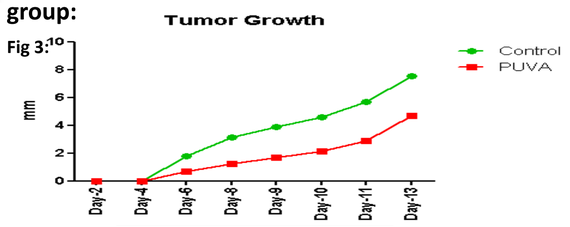
⇒ Immunohistological staining was done to analyze collected tumor tissue.

Statistical comparisons were done using student t-test (GraphPad Prism).

Results

Tumor growth curve and area under the curve analysis showed 51.8% reduction in PUVA vs control group:

Fig 3:



	Control	PUVA
Baseline	0.0	0.0
Total Area	22.82	10.99

Figure 3: Tumor growth curve showed a reduction in tumor development in PUVA-treated mice and during the time period of 2 weeks after EL-4 cell injection. The area under the curve was reduced by 51.8% (i.e. from 22.8 to 11.0 mm2) compared to untreated animals.

PUVA reduces CD4 & CD8+ T-cells in lymph nodes, spleen and blood:

Fig. 4:

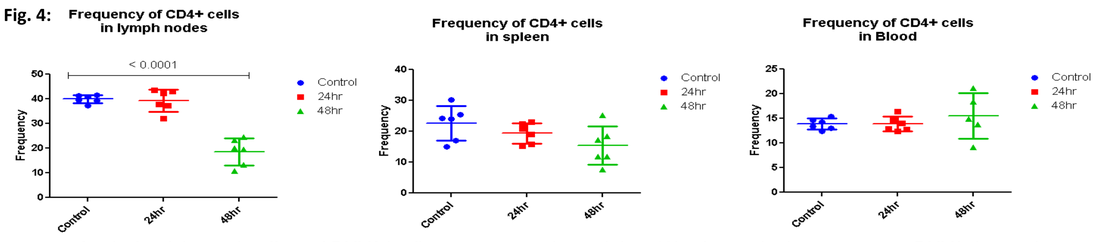


Figure 4: Analysis of the early effect of PUVA by flow cytometry showed that 48h after a single exposure to PUVA the total percentage of CD4+ T-cells was reduced by 53.4% in lymph nodes but not significantly in spleen and blood.

Fig. 5:

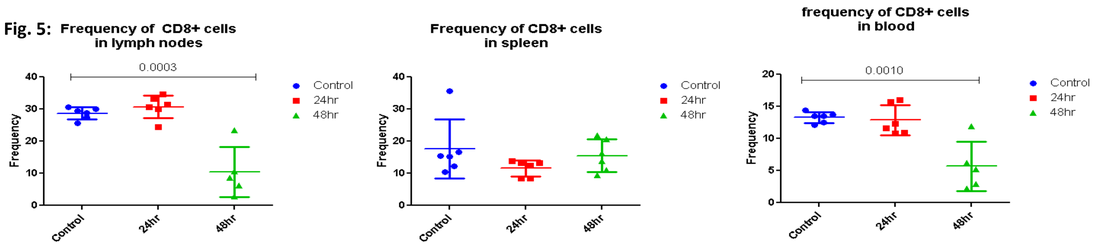


Figure 5: CD8+ T-cells were reduced by 57.2% in blood and 63.7% in lymph nodes (from 13.2 to 5.6 and from 28.7 to 10.3, respectively).

PUVA increases T regulatory cell (Treg) population in blood:

Fig. 6:

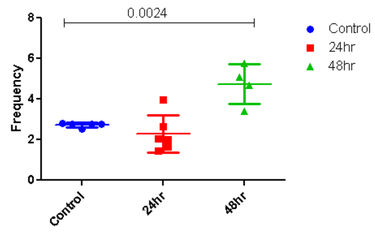


Figure 6: The percentage of Tregs increased in blood of PUVA-treated mice from 2.7% to 4.7%.

PUVA increses the expression of the MHC class II negative regulator LAG-3:

Fig. 7:

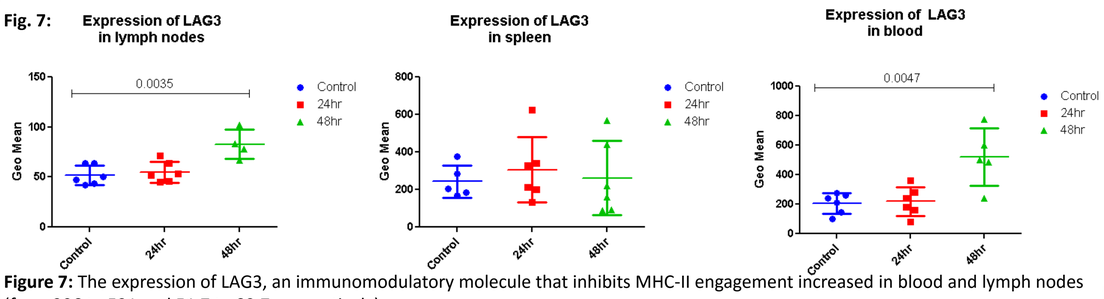


Figure 7: The expression of LAG3, an immunomodulatory molecule that inhibits MHC-II engagement increased in blood and lymph nodes (from 206 to 521 and 51.7 to 82.7, respectively).

PUVA induces clearance of malignant cells in a band-like zone of the superficial dermis :

Fig. 8:

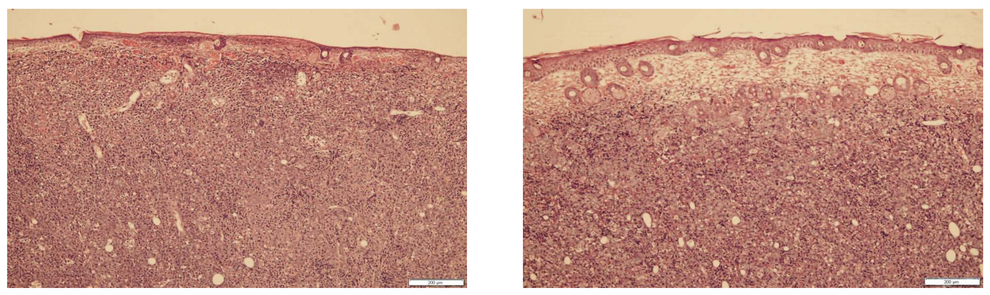


Figure 8: In tissue samples obtained 2 weeks after EL-4 cell injection, histological analysis of tumorous skin showed clearance of malignant cells in a band-like zone of the superficial dermis adjacent to the epidermis and a decrease of overall epidermal thickness in PUVA-treated mice. Scale bars, 200 µm.

Conclusion

- Our mouse model recapitulates the hallmarks of the therapeutic effect of PUVA in CTCL by reducing tumor development and partially clearing the malignant cells from the tissue.
- PUVA therapy triggered a reduction of systemic levels of CD4+ and CD8+ T-cells as well as an increase of Tregs.
- The upregulation of LAG3 in PUVA-treated mice suggests an important role of this molecule in the immunomodulatory effect of this therapy.
- Together these results highlight the involvement of immunomodulation in the therapeutic mechanisms of PUVA in CTCL by easing the tumor burden in lesional skin, possibly by affecting LAG3 and Tregs.

These results suggest that the immunomodulatory effects of PUVA on T cells and their role in therapeutic effect of CTCL.

Acknowledgement

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References:

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