**Abstract**

Most cancer immunotherapies (IT) have a higher likelihood of succeeding if the targeted tumor has a preexisting state of immunomodulation elicited by the combined presentation of shared- and neo-antigens from tumor cells. Thus, novel combination treatment modalities are needed to convert non-immunogenic, ‘cold’ tumors into immunogenic ‘hot’ tumors.

Gp96-Ig/Fc-OX40L is a re-engineered molecular chaperone, designed to export and deliver MHC I-associated antigens to APCs in context of the immune costimulator, OX40L. Allogeneic vaccine cell lines designed to co-secrete Gp96-Ig and Fc-OX40L, generate antigen-specific CD4+CD8+ anti-tumor responses in both highly immunogenic (CT26) and less immunogenic (B16) mouse tumors (Fromm et al., Cancer Immunol. Res. 2016). Such a strategy allows for Gp96-Ig-mediated chaperoning of antigens from the allogeneic vaccine cell line (shared antigens), which could benefit further from increased presentation of tumor-derived peptides (neo-antigens) that are only accessible if Gp96-Ig/Fc-OX40L is expressed from within the tumor. To achieve this, we have employed an in vivo electroporation-based strategy (EP) to deliver Gp96-Ig/Fc-OX40L expressing DNA to tumor cells in situ. Here we set out to determine whether a combination approach of intratumoral EP of Gp96-Ig/Fc-OX40L DNA vaccination and with allogeneic cells co-secreting the same effector molecules would lead to enhanced CD4+CD8+ T cell cross-priming to tumor neo-antigens and superior anti-tumor activity over the individual approaches in a non-immunogenic B16 tumor model.

The combination approach lead to an increased expansion of antigen-specific CD8 T cells in tumors and in the peripheral blood compared to the individual monotherapies, which increased anti-tumor response rates. These findings suggest that a combination approach of allogeneic vaccination and in situ tumor EP of Gp96-Ig/Fc-OX40L, may have significant benefit in eliciting a potent immune response in less-immunogenic tumors.

**Optimization of Electroporation (EP) Conditions For Gp96-Ig/Fc-OX40L DNA Delivery into B16.F10-ova melanoma tumors**

- **A** Electroporation parameters
  - Voltage: 200
  - Number of pulses: 40
  - Pulse duration: 30 ms
  - Gap time: 600 ms

- **B** Gp96 Ig/Fc-OX40L protein levels in tumor
  - EP only vs Gp96.Ig/Fc-OX40L + EP = 0.006

- **C** Gp96.Ig/Fc-OX40L + EP
  - EP only vs Vaccine control = 0.003

**Combination of Intratumoral EP and Allogeneic Vaccination of Gp96-Ig/Fc-OX40L Leads to Increased Expansion of CD8 T Cell Cross Priming and Improved Anti-Tumor Response**

- **A** Intratumoral EP of Gp96-Ig/Fc-OX40L DNA Alone Leads to CD8 T Cell Cross Priming and Delayed Tumor Growth
  - Ununated tumor (control)
  - EP only
  - Gp96.Ig/Fc-OX40L EP only
  - mRNA levels in tumor (tumor lysate associated OX40L protein was quantified by flow cytometry reveals increased numbers of CD8+ T cells positive for Nk1.1, Gr-1, CD11b and CD11c and subsequently pre-gated on CD3+.

- **B** Overall survival
  - EP only vs Gp96.Ig/Fc-OX40L + EP = 0.003

**Key Points**

- In vivo EP of DNA-based Gp96-Ig/Fc-OX40L into B16 melanoma tumors result in CD8 T cell cross priming, increased antigen-specific precursor memory T cells and delayed tumor progression of treated and distal, untreated tumors.

- Combining in vivo electroporation of Gp96-Ig/Fc-OX40L DNA with an allogeneic vaccine secreting the same therapeutic agents enhances the efficacy of treating B16 melanoma tumors due to improved CD8 T cell cross priming.

- This study provides proof-of-concept for pairing Gp96-based vaccines with intratumoral Gp96 therapies

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