Intratumoral Administration of a Multigenic Construct by Electroporation Can Effectively Modulate Anti-Tumor Response in a MURINE B16.F10 MODEL

Background: Immunomodulatory cytokines, such as IL-12, are attractive candidates for cancer immunotherapy. IL-12 is a pro-inflammatory cytokine with potent anti-tumor effects; however, systemic administration shows limited clinical efficacy and dose-associated toxicity. In preclinical and clinical studies, intratumoral (IT) delivery of IL-12 plasmid DNA by electroporation (EP) can be a safe and effective alternative for efficacious dosing. To augment the effects of IL-12, we developed a DNA plasmid platform that allows for delivery of agents that modulate multiple immune pathways as well as tumor- or patient-specific neoantigens. Polycistronic I: a) Multicistronic Igmplasmid (PIIM) is a single plasmid encoding IL-12, and a fusion of Flt3L to an antigen. Flt3L is a ligand that stimulates dendritic cell maturation and enhances antigen processing and presentation. The encoded antigen can be a viral or shared antigen, or a patient-specific neoantigen, which enables customization to patient populations, as well as providing an aid to monitoring antigen-specific immune responses(s) that can be correlated to patient outcomes. Here we demonstrate the first functional characterization of PIIM.

Materials & Methods: Two PIIM constructs were created for functional characterization: PIIM-OVA (IL12-Flt3L-OVA) for mouse experiments, and PIIM-NYESO1 (IL12-Flt3L-NYESO1) for testing in human cells. The expression and functional activity of PIIM components were determined. Treated tumors and spleens were assessed for transcriptional changes by NanoString® and phenotypic changes by flow cytometry. Systemic effects of PIIM were assessed using a syngeneic two-tumor model of B16.F10 in which only one tumor received IT-EP while the other contralateral lesion remained untreated. Results: PIIM-OVA and PIIM-NYESO1 secretes functional IL-12, Flt3L-OVA and Flt3L-NYESO1 fusion proteins as assessed by ELISA, flow and cell-based assays. PIIM promotes DC maturation and antigen-specific T cell proliferation both ex vivo and in vivo. Hydrodynamic-based gene delivery, specifically IT-EP, was both tumor and cellular toward PIIM-OVA and PIIM-NYESO1. Furthermore, IT-EP-PIIM lead to generation of specific OVA-specific CD8+ T cells. When introduced intratumorally in a mouse two-tumor model, IT-EP-expressed Flt3L-OVA, enhanced anti-tumor efficacy. A combination of functional immune modulators can be expressed locally to enhance adaptive immunity and addressing patient-specific neoantigens needs.

Expression of IL-12p70 and Flt3L-antigen co-expressed from PIIM plasmid are functional

**IL-12p70 and Flt3L-antigen co-expressed from PIIM plasmid are functional**

**a)** IL-12p70 Reporter Cells

- pIL-12-P2A
- HEK-Blue IL-12 cells (Invivogen; hkb-il12) produce secreted embryonic alkaline phosphatase (SEAP) upon IL-12p70 signaling via a STAT4 reporter. Dose-response of IL12p70 derived from PIIM expressed and secreted by HEK293 cells following transient transfection with pFlt3L-NYESO1 was assessed by transient transfection in HEK293 and quantitated with a biotinylated anti-hFlt3L antibody and streptavidin-Alexa Fluor 488 (representative flow cytometry graphs, n=3; transfection control is shown as gray dotted line).

**b)** Thp-1 cell binding assay

- PFIGM
- Flt3L-NYESO1-Flt3L-OVA
- PFIGM-R
- IL-12p70
- pIL12-P2A
- HEK293 supernatants

**c)** Maturation and Antigen-specific T cell proliferation

- PFIGM
- Flt3L-NYESO1-Flt3L-OVA
- PFIGM-R
- IL-12p70
- pIL12-P2A
- HEK293 supernatants

**Electroporation of PIIM-OVA generates SiINFEK Ligand C-DC T cells**

**a)** Mouse PIIM-OVA (mIL12-Flt3L-OVA) construct schematic

- mIL12
- Flt3L
- OVA
- Empty plasmid vector

**b)** Generation of SiINFEK Ligand C-DC T cells

- Untreated
- Empty plasmid vector

**c)** Quantitation

- Graph showing IL-12p70 and Flt3L-antigen expression in vitro and in B16.10 tumors

**b)** Electroporation results in B16.10 tumor growth delay

- Primary (treated) lesion
- Contralateral (untreated) lesion

**Summary and Conclusions**

**Hypothetical Model**

- Primary Mouse DCs matured with pFlt3L-OVA present antigen to co-cultured OT-1 T cells
- Mature Mouse DCs MFI
- Induction in expression of antigen presentation machinery genes
- Hydrodynamic delivery of PIIM-OVA to primary tumor
- In vitro expression of Flt3L-antigen
- Intratumoral electroporation:
  - Antigen Presentation Machinery
  - Empty Vector
  - Flt3L-Ag
  - PIIM-NYESO1

- Summary: PIIM represents a molecular platform using the DC maturation factor IL-12, fused to an antigen-specific shared, or patient-specific neoantigen, which can be expressed in a single DNA plasmid, along with potentially immunomodulatory cytokines IL-12p70.