**ABSTRACT**

Imprime PGG® is a soluble pharmaceutical-grade yeast-derived 1,3/1,6 β-glucan being developed for the treatment of cancer in conjunction with anti-tumor monoclonal antibodies (MAbs). Numerous pre-clinical studies in vivo have demonstrated that Imprime PGG redirects innate immune cells, to kill antibody-targeted tumor cells through a complement receptor 3-dependent cellular cytotoxicity (CR3-DCC) mechanism, thereby enhancing anti-tumor and long-term survival effects. Mechanistic studies of the combination therapy in mice have shown that for CR3-DCC to occur, Imprime PGG must be capable of inducing the classical pathway of complement activation and opsonization of tumor cells with IgG, and complement receptor 2 (CR2) on B cells is required for complement activation. Mechanistic studies so far have demonstrated that Imprime PGG-induced antitumor activity in mice requires, at least in part:

- Complement protein C3
- Complement receptor 3 (CR3)
- Complement receptor 2 (CR2) on B cells
- Complement receptor 2 (CR2) on neutrophils

**BACKGROUND**

Imprime PGG, a yeast-derived pharmaceutical-grade soluble 1,3/1,6 β-glucan is being developed for the treatment of cancer in conjunction with anti-tumor MAbs.

- **To-date,** Imprime PGG has been used in 8 trials enrolling >500 subjects. Current clinical development continues in multiple Phase non-small cell lung cancer trials and a Phase 3 colorectal cancer trial.
- **β-glucans** are conserved microbial structures found in the cell wall of unicellular and multicellular pathogens. They are considered pathogen-associated molecular patterns (PAMPs) recognized by the innate immune systems.
- **Yeast-derived β-glucans** have a linear 1,3-β-linked glucan backbone and side-chains, with each side-chain occurring at 1,6 glucan links (Figure 1).

**OBJECTIVES**

- **Evaluate the role of complement activation pathways in binding of Imprime PGG**
- **Antibody-dependent classical pathway (CP)**
- **Manose-binding lectin (MBL) proteins-dependent lectin pathway (LP)**
- **Alternative pathway (AP)**

**EXPERIMENTAL APPROACH AND DESIGN**

- Non-specifically block the complement activation pathways in human whole blood (WB) by ECTA or ECTA
- ECTA chelates both calcium and magnesium ions blocking CP, LP, and AP
- Mg/EGTA allows optimal complement activation by the AP while inhibiting calcium sensitive CP or LP
- Specifically inhibit the CP, LP, or the AP by blocking MAbs
- Anti-C3 MAbs to block CP
- Anti-MBL MAbs to block LP
- Anti-Factor D MAbs to block AP
- Further confirmation of the role of specific complement activation pathway
  - Effect of C1q-depleted serum on binding
  - Effect of C1q replenishment in the serum on binding

**RESULTS**

**IN VITRO STUDIES IN HUMAN**

In vitro mechanistic studies so far have demonstrated that Imprime PGG by human immune cells, requires at least in part:

- Complement protein C3
- Complement receptor 3 (CR3)
- Innate immune cells

**IN VIVO MECHANISTIC STUDIES IN MICE**

In vivo mechanistic studies so far have demonstrated that Imprime PGG-induced antitumor activity in mice requires, at least in part:

- Complement protein C3
- Complement receptor 3 (CR3)
- Innate immune cells

**SUMMARY**

- Activation of the classical pathway by naturally occurring anti-β-glucan antibodies is critical for complement opsonization and binding of Imprime PGG in both CR3 and CR2 receptors on human immune cells in vitro.
- The alternative pathway has minimal involvement in complement opsonization and binding of Imprime PGG.
- The lectin pathway is not required for opsonization and binding of Imprime PGG.