Chimigen® Malaria Multi-antigen Vaccine: A Novel Dendritic Cell Receptor-Targeted Prophylactic/Therapeutic Malaria Vaccine

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ABSTRACT

Chimigen® Vaccines are a new class of chimeric molecules with functional attributes of both antigen(s) and a xenotypic monoclonal antibody. These molecules target the host immune system using specific receptors on dendritic cells (DCs), are processed and presented on both MHC class I and II, giving rise to robust immune responses involving both $CD8^+$ and $CD4^+$ T cells to elicit cellular and humoral immune responses against specific antigens. This platform technology has been used to design a Malaria Multi-antigen Vaccine targeted against *Plasmodium falciparum* (*Pf*). The Chimigen® Malaria Multi-antigen Vaccine incorporates three engineered *Pf* antigens; circumsporozoite protein (CSP), merozoite surface protein-1 (MSP-1) and apical membrane antigen-1 (AMA-1) as well as hepatitis B core protein and a murine Fc fragment. The fusion protein was produced in Sf9 insect cells using the Bac-to-Bac baculovirus expression system (Invitrogen). The protein has been purified by HisTrap FF FPLC (GE Healthcare) and characterized by western blot analysis using antibodies against different regions of the fusion protein. The glycosylation of the protein was evaluated by ConA-HRP staining. Immunological evaluation was carried out using human PMBC-derived DCs and autologous T cells. Chimigen® Malaria Vaccine bound to immature DCs *ex vivo*. In antigen presentation assays *ex vivo*, the vaccine induced a vigorous $CD4^+$ and low $CD8^+$ T cell proliferation measured by CFSE, following the first exposure of autologous T cells to vaccine-pulsed mature DCs. There was also increased secretion of IFN-γ by $CD3^+$ T cells upon initial exposure to the vaccine. A second exposure of T cells to vaccine-pulsed mature DCs resulted in the enhanced secretion of IFN-γ, TNF-α, perforin and granzyme B by both $CD4^+$ and $CD8^+$ subsets. In addition, the vaccine increased the expression of CD27, CD38 and CD138 in B cells as well as surface expression of IgG and IgM when cultured with T cells. The latter data suggests that naïve B-cells exposed to the vaccine in the presence of T cells are able to differentiate into terminal plasma cells. Taken together, our preliminary data suggests that this novel Chimigen® Malaria Multi-antigen Vaccine elicits humoral and cellular immune responses to *Pf* antigens and therefore shows potential for development as prophylactic/therapeutic vaccine against malaria infection.

INTRODUCTION

Malaria is caused by apicomplexan protozoan parasites of the *Plasmodium* genus which are endemic in 108 countries; with more than 3 billion people being at risk of infection. There are currently an estimated 200-500 million cases of malaria worldwide and 80% of all cases occur in sub-Saharan Africa. Malaria kills between one and three million people annually, and 91% of these deaths occur in sub-Saharan Africa. Pregnant women and children under the age of five are at the highest risk of succumbing to malaria. More than 80% of the deaths in sub-Saharan Africa occur in children under the age of five years.

Five species of *Plasmodium* parasites (*P flaciparum, P vivax, P ovale, P malariae and P knowlesi*) can infect humans. The most common and the deadliest species which infect humans is *P falciparum*. The *Plasmodium* parasite has a complex life cycle, reproducing asexually in humans and sexually in mosquitoes of the genus *Anopheles*. When an infected mosquito takes a blood meal, infectious malaria sporozoites ride into the bloodstream along with the mosquito's saliva and migrate to the liver, where they infect and reproduce asexually inside the hepatocytes. One infected hepatocyte can give rise to tens of thousands of mature merozoites which burst out of the liver cells and migrate into the bloodstream, where each merozoite can infect a red blood cell. Inside the erythrocytes, merozoites evade the immune system while feeding on the cytoplasm and replicating asexually. Every infected erythrocyte bursts, giving rise to dozens more of merozoites which invade fresh erythrocytes, perpetuating and exacerbating the cycle of infection. Each successive round of erythrocyte lysis causes release of TNF-α and other cytokines which trigger sharp increases in body temperature and other symptoms such as shivering, cough, joint pain, headache, diarrhoea, vomiting and convulsions. Loss of infected erythrocytes due to lysis can also lead to severe anemia. As infected erythrocytes adhere to blood vessel walls and obstruct blood flow in various organs, more severe symptoms such as respiratory distress, retinopathy, kidney failure, as well as placental and cerebral malaria can develop. These symptoms can result in death if the patient cannot develop strong immune responses to contain and clear the infection.

There is currently no licensed vaccine for malaria. Traditional approaches to vaccine development have focused on adjuvanted antigens or attenuated pathogens. These approaches have resulted in limited success due either to immune evasion by the parasite or ineffective antigen presentation by DCs and insufficient induction of humoral/cellular immune responses. Effective protective immune responses against malaria infection involve both the generation of neutralizing antibodies to sporozoite and merozoite forms of the parasite in blood, as well as cell-mediated responses against various antigens to clear infected cells. The development of multi-antigen vaccines that target different stages of the life cycle of the parasite is crucial in the development of effective malaria vaccines. Several parasite proteins have recently been proposed as potential candidates for such vaccines. These include circumsporozoite protein (CSP), apical membrane antigen-1 (AMA-1) and merozoite surface protein-1 (MSP-1).

Paladin Biosciences has used its proprietary Chimigen® Platform technology to produce a malaria vaccine incorporating the three malaria antigens (Figure below), and the results of our preliminary evaluation of the vaccine are presented.

Chimigen® Malaria Multi-Antigen Vaccine

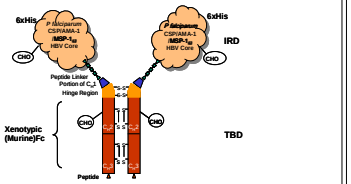

Figure 1. Chimigen® molecule consisting of immune response domain (IRD) (malaria antigens fused to HBV core protein), linked to a xenotypic murine Fc molecule which comprises the terminal binding domain (TBD). The control protein used in this study consists of fused *Pf* proteins shown above without HBV core protein and TBD. Non-mammalian glycosylation (CHO) is also depicted.

Unique Characteristics of a Chimigen® Vaccine

- Fusion protein comprised of antigen(s) (the immune response domain – IRD) and the Fc fragment of a xenotypic monoclonal antibody (the target binding domain - TBD)
- Adaptable platform; can incorporate any relevant antigen
- Unique chimeric design with antigen(s) and antibody properties
- Increased immunogenicity due to the xenotypic nature of the TBD and expression in insect cells which imparts non-mammalian glycosylation
- No added adjuvant
- Effective at low doses (μg)
- The TBD facilitates binding of Chimigen® Vaccines to FcγRII (CD32) and macrophage mannose receptor (CD206) on DCs
- Antigen presentation via MHC class I and class II pathways
- Generates both cellular and humoral immune responses
- Can be used for producing prophylactic/therapeutic vaccines

RESULTS

Chimigen® Malaria Vaccine Has Been Expressed, Purified And Characterized

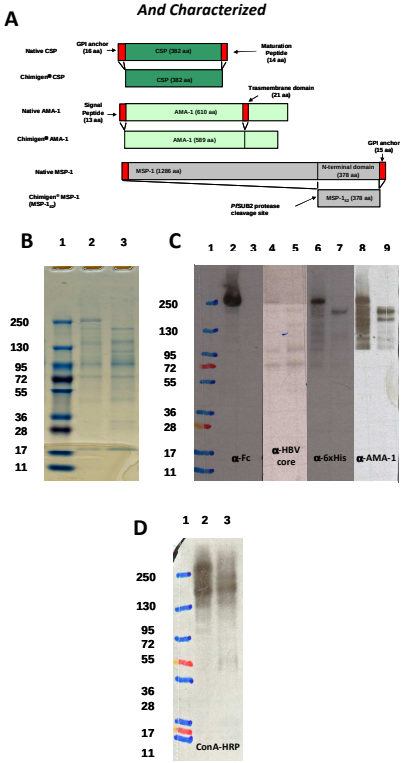

Figure 2. **A)** Design of *Pf* Chimigen® Multi-antigen Malaria Vaccine. The engineered DNA sequences were codon-optimized for expression in insect cells and synthesized by GenScript (Piscataway, NJ). **B)** The fusion protein was purified using HisTrap FF and characterized by PageBlue staining (Fermentas). **C)** Western blots using antibodies specific to different regions of the vaccine. Antibodies used are listed at the bottom of the blots. **D)** Glycostain of Chimigen® Multi-antigen Vaccine. In panels B, C and D, Lane 1 contains PageRuler plus MWM (Fermentas). For all the blots, even numbered lanes are loaded with Chimigen® Multi-Antigen Malaria Vaccine and odd numbered lanes are loaded with control Antigens (no HBV core, no TBD).

Chimigen® Malaria Vaccine Binds to Immature DCs

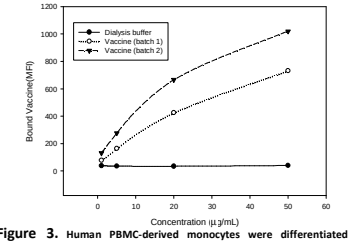

Figure 3. Human PBMC-derived monocytes were differentiated into immature DCs by the addition of GM-CSF and IL-4 for 24 h and used in the binding assay. PBMC-derived immature DCs were incubated for 1 h at 4°C with 2 different batches of Chimigen® Vaccine (1-50 μg/mL). Bound protein was detected by flow cytometry following labeling with biotinylated anti-mouse IgG mAb and streptavidin-PE-Cy5.

Assessment of T Cell Responses to the Chimigen® Malaria Vaccine – Antigen Presentation Assay (APA)

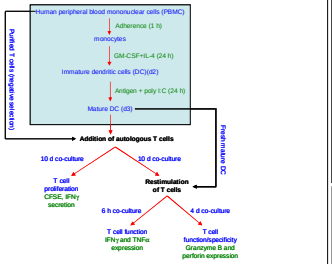

Figure 4. Monocytes and autologous T cells were derived from healthy donor PBMCs. Immature DCs were derived from purified monocytes by the addition of GM-CSF and IL-4 for 24 h. For APA, immature DC were loaded with either Vaccine or its individual components and matured with Poly I:C for 24 h. Mature DCs were incubated with autologous T cells for 10 days. T cell proliferation and IFN-γ secretion were measured following the first stimulation of T cells by mature DCs. Expanded T cells were then re-stimulated with vaccine-loaded mature DCs for 6 h or 4 days and T-cell function was assessed.

Chimigen® Malaria Vaccine Induces Proliferation of $CD3^+$ T cells ($CD4^+$ & $CD8^+$)

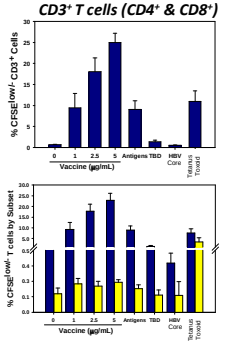

Figure 5. Proliferation of T cells following first stimulation with Vaccine or Vaccine component-loaded mature DC. CFSE-labeled PBMCs were incubated with antigen loaded mature DC for 10 days. Cells were harvested and the degree of proliferation was assessed by loss of CFSE staining using flow cytometry. **A)** Total T cell population ($CD3^+$), **B)** $CD4^+$ and $CD8^+$ T cells. In all figures, individual components of the Vaccine used as controls are either fused *Pf* Antigens (Antigens), target binding domain (TBD) or HBV Core protein. Tetanus toxoid was used as a positive control for T cell activation.

Chimigen® Malaria Vaccine Induces Th1 Cytokines IFN-γ and TNF-α Production in $CD3^+$ T Cells ($CD4^+$ and $CD8^+$)

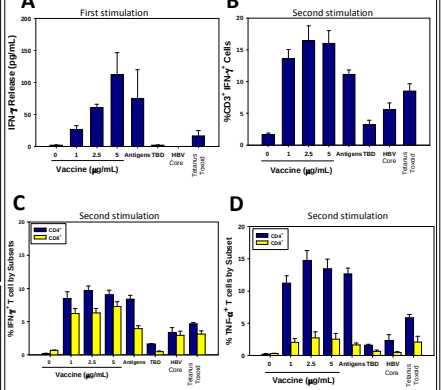

Figure 6. Vaccine-induced IFN-γ and TNF-α production by T cells. **A)** PBMCs were co-cultured with Vaccine or Vaccine component-loaded mature DC for 10 days. IFN-γ secretion by T cell population was assessed by ELISA. **B)** $CD3^+$ cells expanded by first stimulation were co-cultured with Vaccine or Vaccine component-loaded mature DCs for 6 h and cytokine production was assessed by intracellular staining. **C)** T cell subsets expressing IFN-γ and **D)** TNF-α following second stimulation (intracellular stain).

Chimigen® Malaria Vaccine Induces Granzyme B Production in $CD3^+$ T Cells ($CD4^+$ & $CD8^+$)

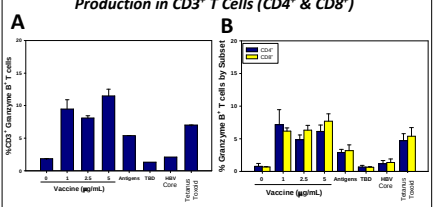

Figure 7. Granzyme B production by T cells. PBMCs were incubated with Vaccine or Vaccine component-loaded mature DC for 10 days, then re-stimulated with fresh Vaccine or Vaccine-component-loaded mature DC for 4 days. Granzyme B production in T cells was determined by intracellular cytokine staining and flow cytometry. **A)** Granzyme B expression in $CD3^+$ T cells. **B)** Granzyme B expression in $CD4^+$ and $CD8^+$ T cells.

Chimigen® Malaria Vaccine Induces Perforin Production in $CD3^+$ T Cells ($CD4^+$ & $CD8^+$)

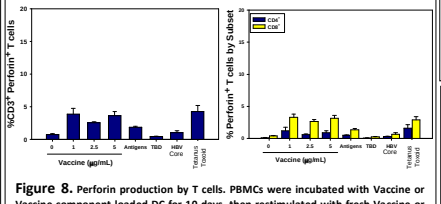

Figure 8. Perforin production by T cells. PBMCs were incubated with Vaccine or Vaccine component-loaded DC for 10 days, then restimulated with fresh Vaccine or Vaccine-component-loaded DC for 4 d. Perforin production in T cells was determined by intracellular cytokine staining and flow cytometry. **A)** Perforin expression in $CD3^+$ T cells. **B)** Perforin expression in $CD4^+$ and $CD8^+$ T cells.

Chimigen® Malaria Vaccine Induces T Cell-Dependent B Cell Maturation Into Terminal Plasma Cells and Up-Regulation of IgG and IgM Expression

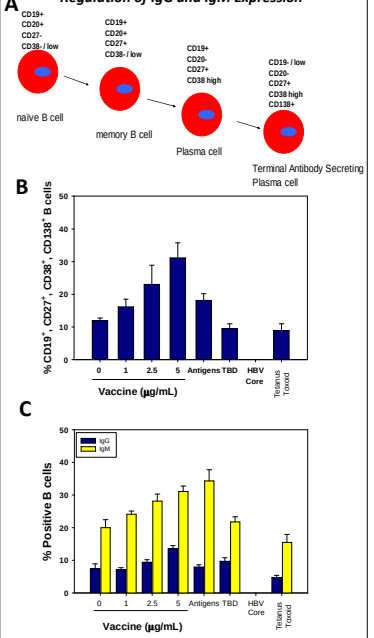

Figure 9. Vaccine-induced T cell-dependent B cell maturation. **A)** Schematic of markers expressed by B cells at different stages of differentiation. B cells were incubated with the vaccine in the presence of autologous T cells for 21 days. Following this incubation period, the percentage of B-cells expressing **B)** plasma cell markers and **C)** IgG or IgM surface immunoglobulinins were determined by flow cytometry.

CONCLUSIONS

- The Chimigen® Malaria Multi-antigen (CSP/AMA-1/MSP-1/HBV core/TBD) Vaccine has been cloned, expressed and purified
- The Vaccine binds to immature DCs
- The Vaccine induces proliferation of $CD4^+$ and $CD8^+$ T cells *ex vivo*
- Vaccine-treated mature DCs induce IFN-γ secretion from $CD3^+$ T cells following first stimulation
- Vaccine-treated mature DCs induce IFN-γ and TNF-α expression by $CD4^+$ and $CD8^+$ T cells following re-stimulation.
- The Vaccine induces B cell differentiation into plasma cells
- The T cell immune responses demonstrated in the *ex vivo* assays suggest that the Chimigen® Malaria Multi-Antigen Vaccine is a promising therapeutic/prophylactic vaccine for the treatment of *P falciparum* infections

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