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Communication

Effective Delivery of Cancer Vaccines with Oxidatively Photo-Inactivated Transgenic *Leishmania* for Tumor Immunotherapy in Mouse Models

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Abstract

The parasitic protozoa in the genus of *Leishmania* have exceptionally favorable attributes for exploitation as a vehicle for safe and effective delivery of transgenically incorporated vaccines against infectious and malignant diseases. A dual suicidal mechanism was installed in *Leishmania* via genetic and chemical engineering *in vitro* for accumulation of photosensitizers, rendering them sensitive to dim light for inactivation. *Leishmania* so inactivated are non-viable, but immunologically competent to deliver vaccines for immune-

prophylaxis and –therapy. We have begun to explore the utility of these *Leishmania* for immunization against tumors in experimental mouse models.

Keywords
Leishmania; transgenics; photodyanmic inactivation; vaccine delivery; immunotherapy; tumor

Antigen-specific vaccination remains to be an option for tumor immunotherapy. Delivery of vaccines for this approach includes the strategies of using attenuated bacterial and viral constructs, e.g. *Listeria* [1, 2] and Vaccinia [3]. *Leishmania* are parasitic protozoa, which are uniquely favorable for use as a universal platform to deliver vaccines for disease prevention and therapy [4, 5]. Of particular interest are human cutaneous *Leishmania*, which causes innocuous, self-resolving skin infection [6]. Life-long immunity develops after its spontaneous cure, indicative of not only the presence of *Leishmania*-specific vaccine molecules against leishmaniasis but also adjuvanticity critical for effective vaccination against this and other diseases, e.g. malignancy. *Leishmania* are equipped with eukaryotic translational machineries and post-translational mechanisms for correct expression of multiple transgenic vaccines in abundance, thereby enabling them to serve as a carrier of high efficiency. Additionally, *Leishmania* are endowed with multifarious molecules to protect endogenous vaccines and target them specifically to antigen presenting cells (APC), i.e. dendritic cells (DC) and macrophages – the exclusive host cells for the residence of these parasites in natural infection. Attributable to these vaccine-protection and APC-homing properties are their surface lipoglycoconjugates, contributing to *Leishmania* adjuvanticity for effective vaccination. *Leishmania* are intrinsically safe. They produce no toxins [7, 8] and show no human toxicity when used extensively after chemical or physical inactivation of whole cells in Leishmanin skin test for delayed type hypersensitivity and in several large scale vaccine trial attempts [6].

We have developed novel strategies to completely inactivate *Leishmania* to ascertain their safety with the preservation of its adjuvanticity as a vaccine carrier, viz. their genetic and chemical engineering *in vitro* to install light-activated duo suicidal mechanisms. This is made possible by partial genetic complementation of their deficiencies in heme biosynthetic enzymes for cytosolic accumulation of UV-sensitive uroporphyrin [9, 10] and by loading of their endosomes exogenously with red-light sensitive cationic phthalocyanine [11-13]. Brief illumination of these photo-sensitized *Leishmania* results in their rapid oxidative inactivation initiated by the generation of extremely short-lived, albeit highly destructive singlet oxygen [14]. The safety and efficacy of such inactivated *Leishmania* have been demonstrated by immunization of animals, producing neither infection nor adverse effects [15], but prophylactically protect them against both cutaneous and visceral leishmaniasis [16, 17], and immunotherapeutic activities clinically against drug-incurable canine leishmaniasis [18]. Moreover, *Leishmania* transgenically made to express ovalbumin (OVA) was shown to effectively deliver this antigen, after photodynamic inactivation, to DC for processing and presentation to activate OVA epitope-specific T cells in vitro [12]. Human cancer vaccine candidates have been successfully expressed in transgenic *Leishmania*, including enolase 1 (hENO1) [19]. These inactivated *Leishmania* produced impressive activities of immunotherapy by suppressing the emergence of tumors, which were pre-established with murine and human lung

cancer cells in mice [20]. In one model, frozen samples of photo-inactivated *Leishmania* were used and found more effective than CpG ODN as adjuvants for immunizations with recombinant ENO1 peptides against murine tumor. In another, photo-inactivated hENO1-expressing *Leishmania* were used alone for immunizations of BALB/c mice followed by adaptive transfer of immunity via their splenic cells to immunocompromised mice bearing human tumor. Work is under way to assess such photodynamic vaccination in another murine model for pancreatic cancer. Of interest is to study the mechanism of adjuvanticity of photo-inactivated *Leishmania* in detail for comparison with other DAMP and PAMP adjuvants. Tumor antigens have been delivered to patients' DC via conjugation with cell-penetrating peptides [21] and adeno-associated virus vectors [22] for *ex vivo* vaccination to activate CD8+ T cells for CTL activities of anti-tumor immunity. Such protocols are being developed for use with inactivated hENO1-*Leishmania* toward DC-based immunotherapy of human lung cancer.

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Author Contributions

Conceptualization & writing, KP Chang; Funding Acquisition & Supervision, KP Chang, DKP Ng, RB Bachu, CK Fan; Methodology & Investigation, BK Kolli, RB Bachu, DKP Ng.

Competing Interests

The authors have declared that no competing interests exist.

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